# A STUDY OF CERTAIN ASPECTS OF AMMOCOETE BIOLOGY

T H E S I S

for the

Degree of Doctor of Philosophy

in the

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By

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PREFACE:

Larval lampreys, or ammocoetes, have long been popular subjects for biological research both in Europe and America. Several factors have contributed to this interest. Firstly, ammocoetes can be obtained in large numbers with comparative ease in numerous rivers and streams. Secondly, since Schneider (1879) proved con-:clusively that ammocoetes undergo a complex metamorphosis to become adult lampreys, they have been regarded as affording some insight into the problems associated with the biology and evolution of the early vertebrates.

In North America research on lampreys and ammocoetes has been of a somewhat different character than that done in Europe, and since 1940 this research can be almost entirely attributed to the recently recog-:nized importance of predaceous lampreys to inland fishing industries.

Both of these types of research have omitted to a large extent the natural history of lampreys and ammocoetes as such.

For instance, in Britain ammocoetes have all too often been regarded as little more than biological curiosities to be played about with in the laboratory. The papers which have been written about its reactions to artificial stimuli under highly unnatural conditions (Gribble 1934, Francis and Horton 1936, Stevens 1950, and Harden-Jones 1955) are legion. Several eminent British biologists have concerned themselves with ammocoete and lamprey physiology (Young 1935a and b, Barrington 1942, Roberts 1950, Baxter 1956, Whiting 1957, Morris 1958) thereby contributing data of great value in understanding the natural history of these animals. Hardisty (1944/51) has made a study of ammocoete growth under field conditions, but has dealt with only one species.

On the continent the natural history of both River Lampreys (Lampetra fluviatilis, Linn.) and Brook Lampreys (Lampetra planeri, Bloch) has been extensively studied (Lilljeborg 1891, Smitt 1895, Weissenberg 1925, Lauterborn 1926, and others which will be dealt with later in the text), but these investigations have been concerned only with adult lampreys.

Because of the pressing need for a practical solution to the problem of lamprey control, particularly of land-locked Sea Lampreys in the Great Lakes, North

American research on lamprey biology has dealt principally with such practical considerations as the tolerance of this animal, in its various stages, to certain toxins (Lamsa 1956, Kennedy 1955), electric lamprey barriers (Scott 1956, Johnson 1956, McCauley 1956) and the spread of predaceous Sea Lampreys (Gage 1928, Hubbs and Pope 1937, Creaser 1947, Applegate 1947, East 1949, Shelter 1949, Hile 1951).

Therefore the little information about ammocoete biology that has come from Canada and the United States in recent years has dealt mainly with various species of Brook Lampreys, which are economically unimportant (Dean, Eastman and Sumner 1897, Gage 1898, Young and Cole 1900, and others which are dealt with later in the text).

As a step toward providing some of the badly needed information on ammocoete natural history, the author undertook research on rates of growth in ammocoetes of all three species of lamprey found in Britain (Sea Lamprey *Petromyzon marinus*, River Lamprey *Lampetra fluviatilis*, and Brook Lamprey *Lampetra planeri*).

Before this could be accomplished, it was found necessary to develop an accurate method by which larvae of the three species could be selectively identified. This proved to be a complex problem in itself, and its solution was greatly facilitated by the work of Vladykov (1949) on Canadian ammocoetes.

In the field of ammocoete growth studies itself, some work had already been done, but only on the larvae of Brook Lampreys. Four investigators are important in this regard: L. P. Schultz (1930), Ivanova-Berg (1931), F. G. W. Knowles (1941) and M. W. Hardisty (1944).

Although each of these biologists worked separately on the same species (Brook Lamprey), and al-:though all used essentially the same techniques in order to ascertain the same thing, namely the length of larval life, their conclusions did not agree.

Knowles estimated a larval life of three years, Schultz placed the figure at between three and four years; while Ivanova-Berg came to the conclusion that Brook Lampreys have a larval life of five years. Hardisty's second estimate (1951) agreed with that of Ivanova-Berg, but his first estimate (1944) fell short of this.

Several factors could have contributed to these discrepancies. Hardisty himself pointed out (1951) that there is probably some variation in length of larval life according to stream conditions, etc. However, in view of the fact that different species of lampreys can and do spawn in the same streams and that, until the present author published a key to British ammocoetes, MacDonald (1959), no way was known by which ammocoetes of Brook Lampreys could be distinguished from those of River or Sea Lampreys, it is far more likely that the very great discrepancies discussed in the preceding paragraph, came about as a result of taking more than one species of ammocoete in some of the samples. In this way any at-:tempt at size class analysis would be confounded.

Then, too, each of the four men took their specimens in different countries. In each of the areas concerned the Brook Lamprey parades under a different Latin name, and there may be a significant difference in their lengths of larval life. However, after examining hundreds of specimens of various "species" of Brook Lampreys and their ammocoetes, the author is quite con-:vinced that they all belong to the same species.

Therefore, the author felt that while breaking entirely new ground in making an analysis of rates of growth in Sea and River Lamprey ammocoetes, it was the better part of wisdom to re-analyze L. planeri in this regard. Laboratory experiments were then carried out designed to assess the value of the field work. In conjunction with these experiments, observations were made on certain aspects of ammocoete biology which might be concerned in growth of the animal.

For these reasons the thesis falls into three main divisions:

(a) Identification of ammocoetes of British lampreys.

(b) A study of spawning requirements of adult lampreys prerequisite to an understanding of the embryology and early life of the ammocoetes.

(c) A section dealing with rates of linear growth and gain in weight of ammocoetes under control and experimental conditions, and an examination of various aspects of ammocoete biology which might be expected to play a part in determining rates of growth. Of major importance in this respect are the experiments through which the author ascertained the lengths of larval life required by the three species concerned.

METHODS:

Several methods have in the past been used in the collection of ammocoetes, including employment of shovels, hand dredge nets, etc., but by far the most successful method is through the use of a portable electric shocker.

For many years electric shockers, or stimulators, have been in use in Canada and the United States for fisheries purposes. To date the most popular ammocoete electric shocker, presently employed by the Fisheries Research Board of Canada, is operated by a gasolene powered motor/generator unit, which supplies an AC of high amperage and voltage. This instrument is portable for short distances (Tibbles 1956).

In the present instance, Dr. W. G. Hartley, of the Ministry of Fisheries, Food and Agriculture was so generous as to lend a battery operated portable electric shocker to the author. Powered by two six volt batteries, this instrument supplies an interrupted DC current pulsed at the rate of 41 cycles/second. Its outstanding advantage over any other shockers previously tried by the author lies in its lightness. The whole apparatus, together with the two electrodes, does not exceed twenty pounds in weight, and can therefore be carried long dis:tances.

Many authorities, including D. P. Scott (1956) have declared that fish and ammocoetes would be attracted to the positive pole of such a machine. Extensive use of the shocker under a wide variety of conditions has con:vinced the author that such is not the case. Both poles stimulate ammocoetes, sometimes simultaneously, and neither can be said to exert any attractive force on them.

In collecting ammocoetes with the shocker, it was found disadvantageous to allow the current to run for more than 30 seconds at a time, with a rest of about a minute between each blast. If the ammocoetes are "over-electrocuted" the blood vessels in the branchial region rupture. This not only makes the ammocoete useless as a living specimen, but so discolours the whole gill region as to render it extremely difficult to identify its species.

The author found that dead material was best preserved in a 4% solution of formalin for a day or so and then transferred to 70% preserving alcohol. If ammocoetes so preserved are left in the sun for long periods of time, the pigmentation becomes very obscure, again rendering species identification difficult.

Maintenance of living ammocoetes in the labora:tory raised no serious problems, and as long as a sub:strate of some sort was provided for the larvae to burrow in, along with an inch or so of water, specimens can be kept in good health for months. Provision of running and/or aerated water assures the welfare of ammocoetes for several years. This has also been found to be the case by other investigators, e.g. Viera (1895), Creaser and Hann (1929), Newth (1930).

Ammocoetes are filter-feeders, so that, except for making certain to include some detritus in with the substrate, it was found that no special care about nourishment of the specimens needed to be taken. The best substrates have been shown to be silt, mud or mud and sand combined (MacDonald 1956).

The care of adult lampreys in captivity proved to be a much more difficult proposition. Baxter (1949) examined this problem and came to the conclusion that cold water, amply aerated, was the main requisite. In the present author's experience, large mortalities were incurred if these rules were not followed. On the whole, Brook Lampreys do not succumb under laboratory conditions so quickly as do River Lampreys.

SPECIES OF BRITISH LAMPREYS:

Lampreys belong to the sub-class Cyclostomata and are characterized by the possession of seven gill-slits on each side, two lateral eyes plus a pineal eye on the dorsal surface of the head. At the anterior end of the body is situated the sucking oral disc and a rasping tongue-like organ armed with "teeth". The lamprey body is long and fusiliform, approximately eel-like in shape.

Three species of lampreys have long been recognized in Britain. These are:

(a) Sea Lamprey (Petromyzon marinus). This is the largest species and spends its adult life as a blood-sucking predator of fish either in the sea or in large lakes. In the former case it is of course very difficult to make an accurate estimate of the amount of damage it does. Under normal conditions Sea Lamprey attain a length of three to four feet. Landlocked Sea Lamprey tend to be somewhat smaller.

(b) River Lamprey or "Lampern" (Lampetra fluviatilis): This species is also a predator of fish in the same way that the Sea Lamprey is. They occur very commonly in inland lakes, but usually the adult life is passed in salt water. An adult length of 12 to 15 inches is average.

(c) Brook Lamprey (Lampetra planeri): The Brook Lamprey is the smallest of the three species, rarely exceed:ing an adult length of 15 centimetres. It is non-predaceous and spends its adult life in the compara:tive obscurity of small burns.

With the great differences in size alone as a guide, it is comparatively easy to identify the adults of the three British species of lampreys. However, the ammocoete or larval stages have long proved baffling in this regard.

AMMOCOETES:

The ammocoetes of all species are similar in gross appearance and habits, and in both these respects are sharply differentiated from adult lampreys. They are microphagous feeders, spending most of their time burrowed in the muds of river bottoms. Like adults, they possess seven gill-slits on each side and a pineal eye on the top of the head. The lateral eyes are not visible in ammocoetes, being covered by a thick layer of dermal tissue. Instead of an oral disc they have a loose flabby hood and two side flaps surrounding a round and "toothless" mouth. Also, in place of a rasping tongue is found a knob of soft flesh from which the tongue arises during metamorphosis, which is referred to as the "Precursor of the Tongue". The larvae are likewise equipped with dorsal fins and a nasohypophyseal opening on top of the head.

Of course, there are a number of obvious dif:ferences in the internal anatomy of adult lampreys and ammocoetes, but none of these is relevant to the present dissertation.

IDENTIFICATION:

As stated earlier, there has been a startling lack of research on the problem of ammocoete identification in Europe. L. planeri and L. fluviatilis have been particularly confusing in this regard in that controversy as to whether or not they are truly separate species has been going on for the past eighty years.

Anton Schneider (1879) considered them as belonging to the same species. Waygel (1875) stated that they might be separate subspecies, but certainly not distinct species. Benecke (1886) referred to both as Petromyzon fluviatilis. Moreau (1881) even went so far as to suggest that L. planeri were juvenile stages of L. fluviatilis. Léger (1924) asserted emphatically that the two were separate species and discussed minor morphological differences of the head and mouth in the ammocoetes of River and Brook Lampreys. He pointed out that, at metamorphosis, L. planeri has relatively smaller eyes than does L. fluviatilis at the same stage of development. Léger (1920 and 1924) also made a study of metamorphosing ammocoetes of P. marinus, noting that they differ from larvae of Brook and River Lampreys in having uniformly grey dorsal and lateral surfaces.

However, despite these attempts, ammocoete systematics have been so neglected in Europe that Whiting (1957 - personal correspondence) stated, "No specific differences are known between the young of the three British species of lamprey".

This has not been the case in North America. Creaser and Hubbs (1922) made a comprehensive survey of the systematics of Holarctic lampreys, then Creaser (1940) carried out a more thorough study of the characteristics of certain small Brook Lamprey (Entosphenus sp.) from the North Eastern United States. The real champion of lamprey systematics, however, has been Vadim D. Vladykov, a Canadian biologist. In 1949 he described the morpho-:logical differences between several species of lampreys found in America, and in 1950 he produced a monograph devoted entirely to ammocoete taxonomy, embracing in his study larvae of two types of Brook Lampreys (Entosphenus lammotenni and Ichthyomyzon aepyptera) and the Sea Lamprey (P. marinus).

The techniques which Vladykov used in identify-:ing American ammocoetes were of great use in guiding the present author in his attempts to identify British ammocoetes. A field key to larvae of the three British species was established (MacDonald 1959). In the present paper this key is amplified and several ammocoete characteristics not included in the 1959 publication are discussed.

In making the present analysis over 11,000 ammocoetes were carefully examined and compared, as were 175 adult lampreys. This latter was done in order to verify myomere counts made on ammocoetes.

Preliminary examination indicated 7 taxonomic characters which seemed to show species variation. These were:

(a) Pigmentation of the precursor of the tongue.

(b) Pigmentation of the caudal fin.

(c) Pigmentation of the head and branchial region.

(d) Pigmentation of the trunk myomeres.

(e) Shape of the caudal fin.

(f) Shape of the snout.

(g) Number of trunk myomeres.

Of course the greatest difficulty to overcome was in being certain of the species of an ammocoete before assessing its characteristics. Until the present, people investigating ammocoetes seem to have been under the impression that all ammocoetes found in a given small area must be of the same species, and that this species could be determined by a sort of guess work based on what adult lampreys had been caught or seen nearby. This attitude has no foundation in fact. In the River Forth, for instance, ammocoetes of all three species were collected in one shovel full of mud. (See Table I).

At the beginning of this study it was assumed that lampreys would have to be bred in captivity before any unquestionable evidence of species differences could be found. Therefore, in December 1957, 23 River Lamprey adults were obtained from the Severn River near Gloucester. These were maintained under as natural con-:ditions as possible in a large aquarium. It was hoped that at least some of them would spawn and provide ammocoetes of a known species. Seven of these lampreys did spawn during the following Spring, and examination of some of the ammocoetes nearly two years later upheld the former tentative conclusions to which the author had arrived through analysis of field samples.

Another proof was obtained by keeping living ammocoetes in tanks, after counting their myomeres and examining their pigmentation, until some of them meta-:morphosed. In this way the data derived from the pre-:liminary examination could be correlated with known species.

The three species were tentatively identified as follows. A strong similarity was noticed between ammocoetes taken from the Endrick River and those of Entosphenus lamottenii, an American species of Brook Lamprey very much like L. planeri. These unidentified ammocoetes from the Endrick were thus hypothetically con-:sidered as L. planeri larvae. Also identification of Sea Lamprey ammocoetes presented no difficulties, as Vladykov (1950) had already developed a key for the species in Canada.

By this time about 900 ammocoetes had been collected and examined, and provided that the tentative identification of L. planeri and P. marinus larvae had been correct, only one group of ammocoetes, all exhibiting similar charactertistics, remained unidentified. They were assumed to be L. fluviatilis.

TABLE I: SOURCES OF MATERIAL

| | | Number of Ammocoetes Taken | | | |
|---|---|---|---|---|---|
| River | Locality | *L. planeri* | *L. fluviatilis* | *P. marinus* | Total |
| Humber | England | 166 | 74 | 45 | 285 |
| Trent | England | 22 | 7 | 0 | 29 |
| Isis | England | 40 | 50 | 0 | 90 |
| Severn | England | 2 | 125 | 866 | 993 |
| Tad | England | 857 | 405 | 0 | 1,262 |
| Ysteth | Wales | 11 | 0 | 0 | 11 |
| Dee | Scotland | 52 | 77 | 0 | 129 |
| Forth | Scotland | 759 | 135 | 12 | 906 |
| Clyde | Scotland | 746 | 2,019 | 206 | 2,971 |
| Inler* | Scotland | 2,281 | 722 | 6 | 3,009 |
| Fruin | Scotland | 328 | 200 | 0 | 528 |
| Endrick | Scotland | 779 | 288 | 49 | 1,116 |
| | | 6,043 | 4,102 | 1,184 | 11,329 |

*A tributary burn to the River Leven, Dunbartonshire, about 1 km. from Loch Lomond.

TAXONOMIC CHARACTERISTICS:

(a) Pigmentation of Precursor of Tongue: The parts of this organ of use in the present discussion are illustrated in Fig. 1. The precursor can be removed for examination by cutting the floor of the ammocoete mouth. In ammocoetes of *L. planeri* the tip, middle and base of the precursor are unpigmented (Fig. 1). Also, both the base and the middle are swollen, giving them a bulb-like appearance. In *L. fluviatilis* the base and middle are pigmented and not bulb-like (Fig. 2). In *P. marinus* the middle region is relatively shorter than it is in either of the other two species. The base is unpigmented and is not bulb-like (Fig. 3). This characteristic is the best for the purpose of segregating species of dead ammocoetes but, of course, it cannot be used with living material.

(b) Pigmentation of the Caudal Fin: On the basis of this characteristic British ammocoetes immediately fall into two groups. Those in which the pigmentary melanophores are scattered widely over the caudal fin, sometimes right to the periphrey; and those in which the pigmentation is

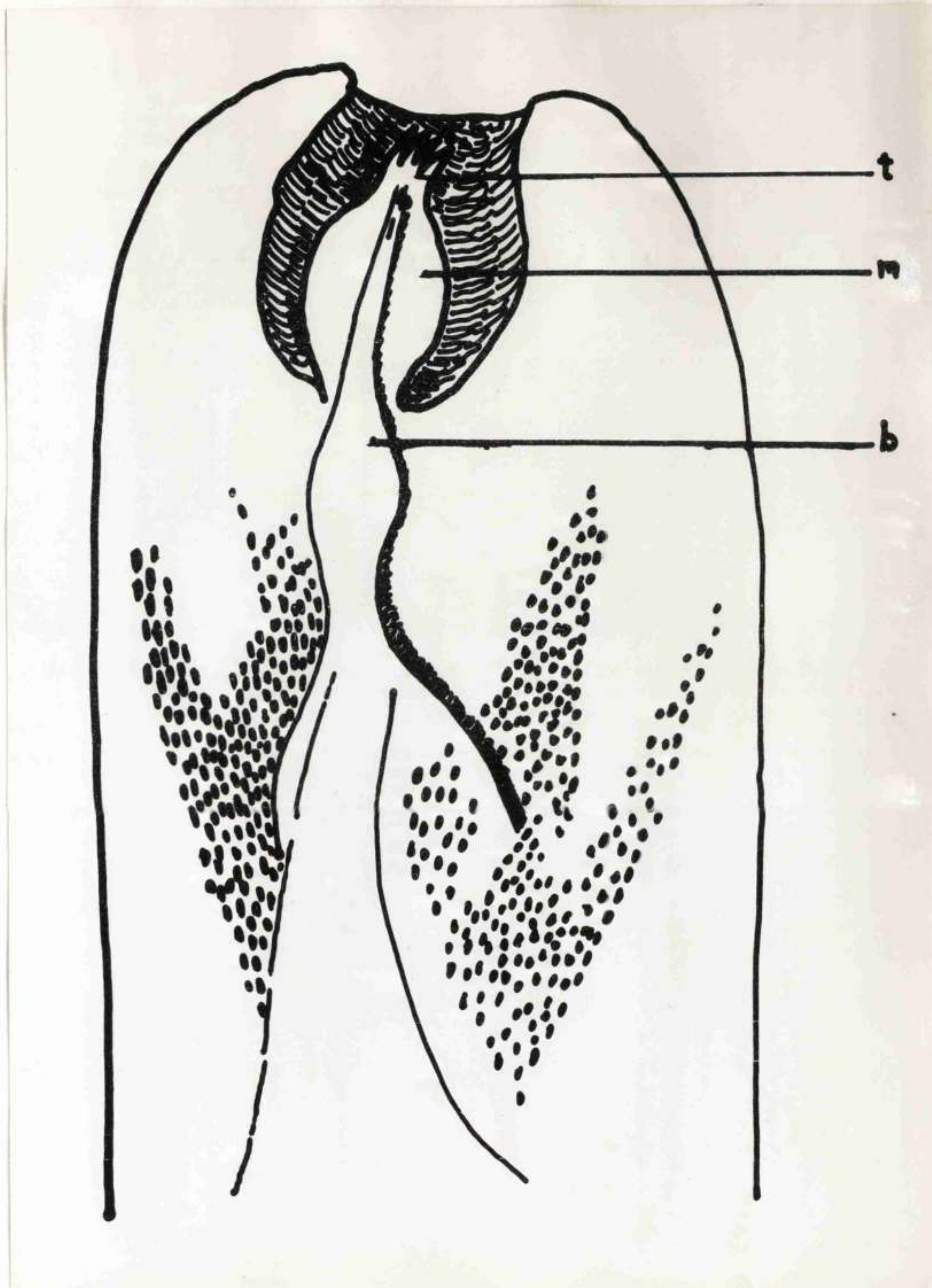


Fig. 1: Precursor of the tongue in L. planeri ammocoete. The regions of taxonomic importance are the tip (t), the middle (m) and the base (b). Note that in this species the tip, middle and base are unpigmented, and that the base is bulb-like. Magnification: 1000X.

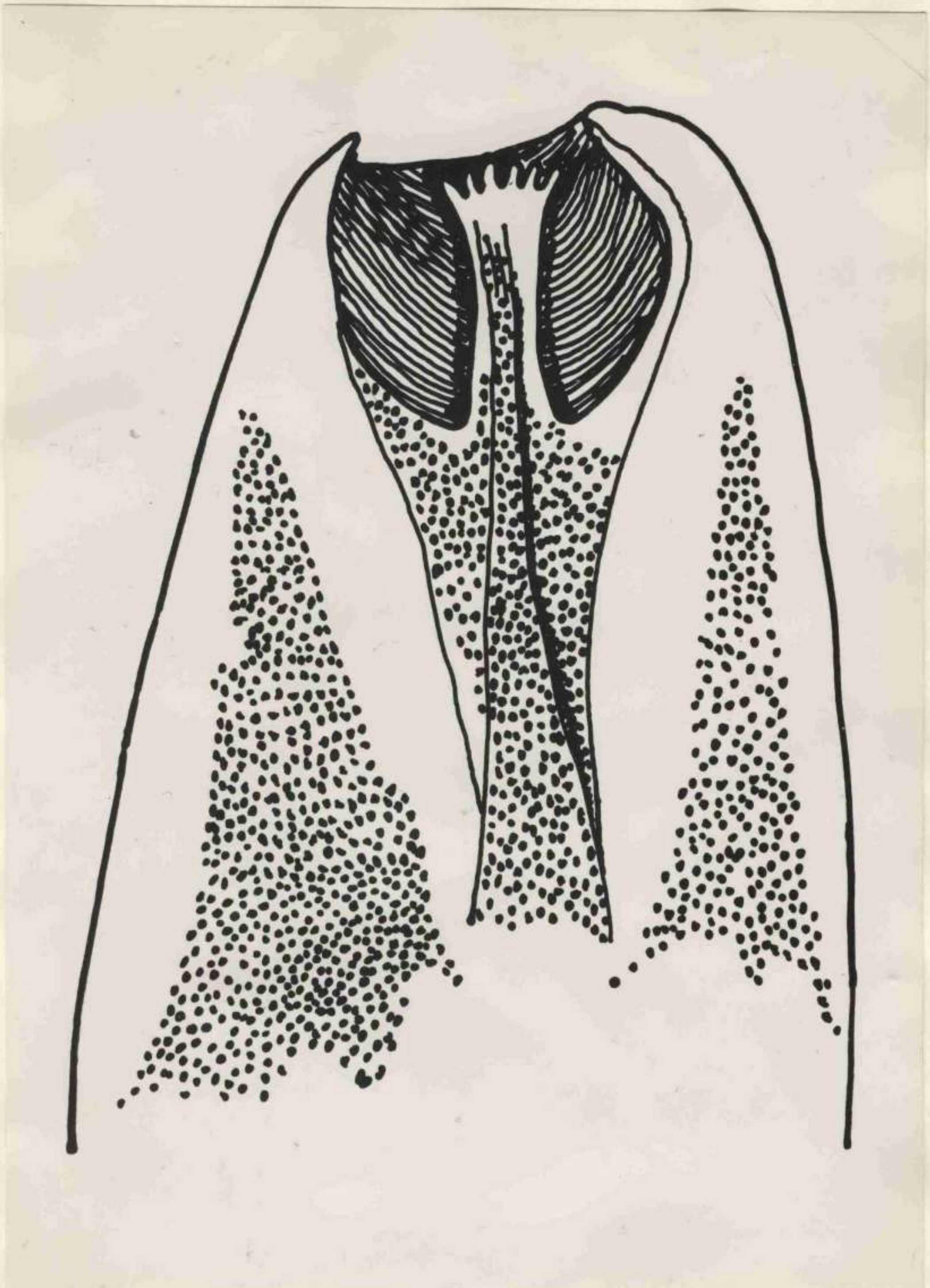

Fig. 2: Precursor of the tongue in L. fluviatilis ammocoete. Note that the base and the middle are pigmented while the tip is not. Magnification: 1000X.

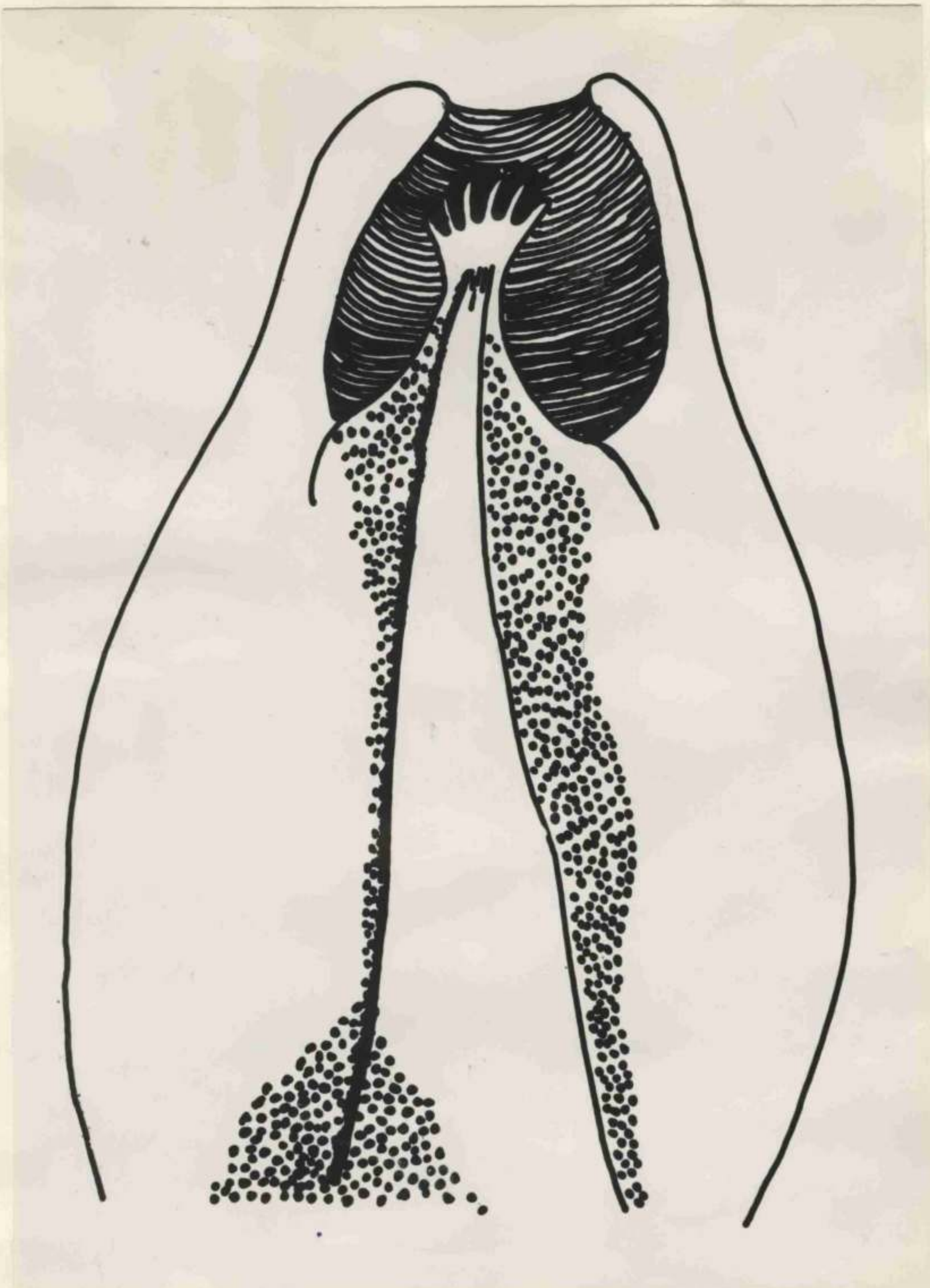

Fig. 3: The Precursor of the tongue in P. marinus ammocoete. Note that the base, middle and tip are unpigmented and that the base is not bulb-like. Magnification: 1000X.

confined to a narrow band outlining the posterior tip of the body. In L. planeri larvae (See Fig. 4) there are relatively few melanophores visible on the caudal fin, and these are small and jet black, and are concentrated in a narrow band enclosing the posterior tip of the body. For this reason the greater part of the area of the caudal fin in this species is completely transluscent and devoid of pigment. In L. fluviatilis the melanophores are large and dark brown, and the field of pigmentation extends from the edges of the body tip, where it is most concen-:trated and hence darkest, out over the main area of the caudal fin. The pigmentation rarely extends to the edges of the caudal fin, but generally presents an irregular edge which does not follow the contour of the outer edge of the caudal fin itself. (See Fig. 4b). In P. marinus ammocoetes the pigmentation extends almost to the edges of the caudal fin, and presents a smooth, regular out-:line, following the edge of the caudal fin. Also the melanophores of this species are grey in colour and intermediate in size between those of L. planeri and L. fluviatilis. (See Fig. 4c).

In all ammocoetes the extent of pigmentation on this part of the body increases somewhat as the larvae

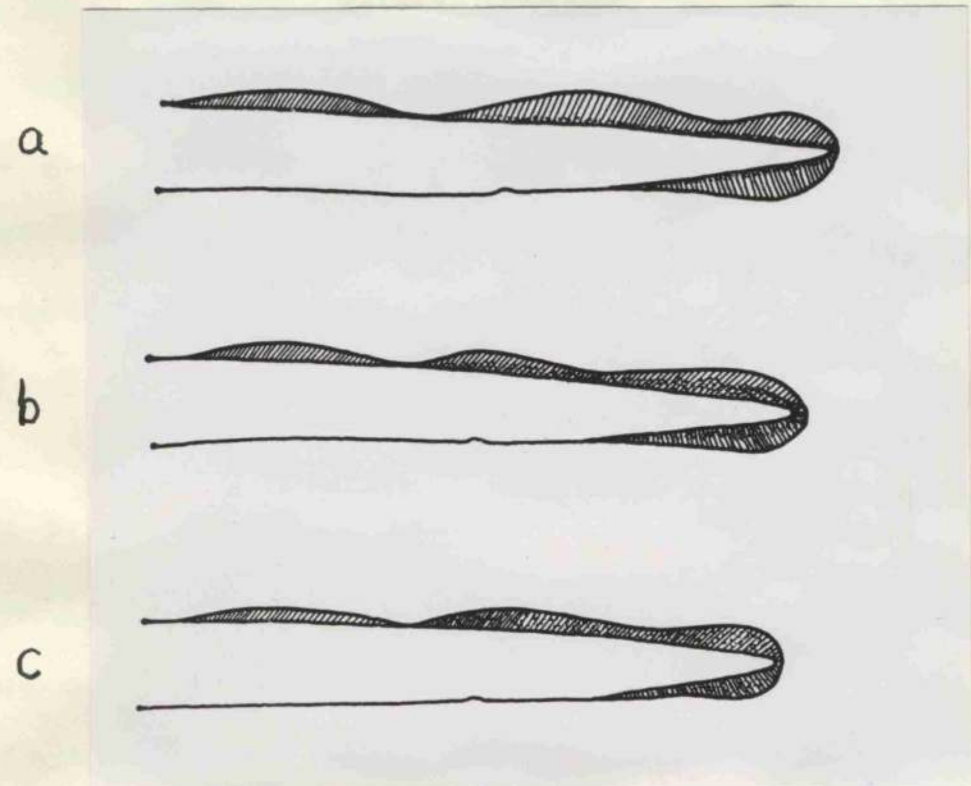


Fig. 4: The posterior ends of ammocoetes.
Magnification: 4.5X.
(a) L. planeri.
(b) L. fluviatilis.
(c) P. marinus.

grow, and this phenomenon is particularly marked in large _L. planeri_ ammocoetes just previous to metamorphosis, which on the basis of caudal fin pigmentation alone, might be confused with _L. fluviatilis_. (See Fig. 5).

(c) _Pigmentation of the Head and Branchial Region_: The branchial region (bounded anteriorally by the crease marking the position of the eye and posteriorally by the 7th. gill-slit) is fairly distinctively pigmented in all three species. In _L. planeri_ and _L. fluviatilis_ ammo-:coetes, all of the pigmentation lies above the row of gill-slits in the branchial region, while in _P. marinus_ larvae pigmentation extends below the row of gill-slits. (See Figs. 6a, 6b, 6c). This characteristic is therefore suitable for separating ammocoetes of _P. marinus_ from those of the other two species. A similar difference can be found in the pigmentation of the snout. In _P. marinus_ the mouth flaps are completely pigmented, whereas in the other species a white unpigmented patch is very prominent in this region.

(d) _Pigmentation of Trunk Myomeres_: The trunk myomeres lie between the 7th. (most posterior) gill-slit and the anus. In _P. marinus_ pigmentation in this region is uni-:form, extending down both sides and on the dorsal

a

b

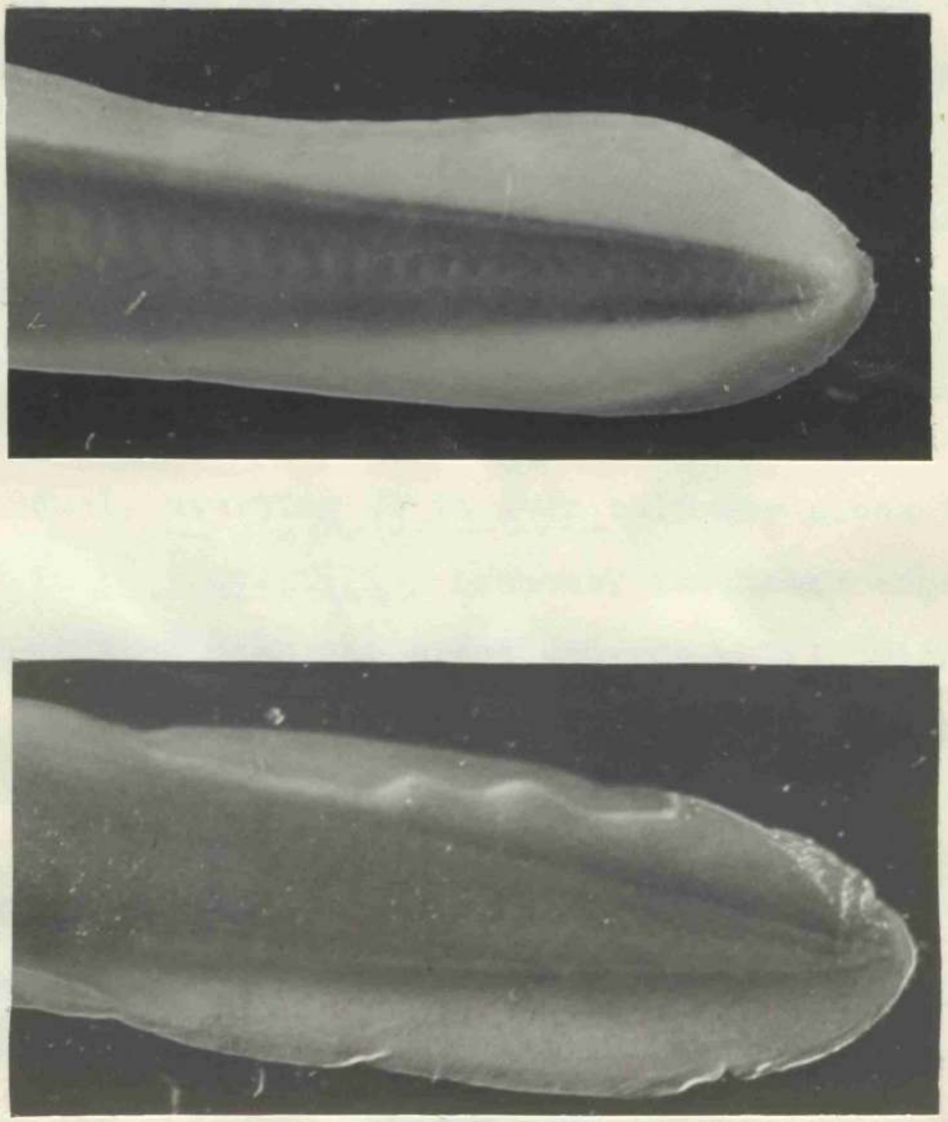

Fig. 5: Caudal fins of L. planeri ammocoetes.
Magnification: 10X.
(a) larva 14 months old.
(b) larva about to undergo metamorphosis.

surface, evenly all the way along. Larvae of both L. planeri and L. fluviatilis have an uneven trunk pig:mentation. From the anus anteriorally until part of the way along the trunk, the pigmentation extends down the lateral surfaces fairly evenly. It then rises, leaving an ever widening unpigmented margin on the lower lateral surfaces of the trunk. The trunk pigmentation unites evenly with that of the branchial region over the gill-slits. In L. planeri the slope of the line of demarcation between pigmented and unpigmented areas is quite gradual, starting as it does half-way along the trunk. In L. fluviatilis, however, the lower edge of the pigmentation does not slant upwards until quite close to the posterior end of the branchial region, so that its junction with the branchial pigmentation is more abrupt than is the case in L. planeri.

(e) Shape of Caudal Fin: The caudal fin of L. planeri is rather deeply notched dorsally at a point about two-thirds of the distance between anus and posterior tip of the body, until just before metamorphosis, when this notch shallows considerably. In L. fluviatilis the notch is always quite shallow, and tends to occur closer to the anus. Ammocoetes of P. marinus are very similar to those

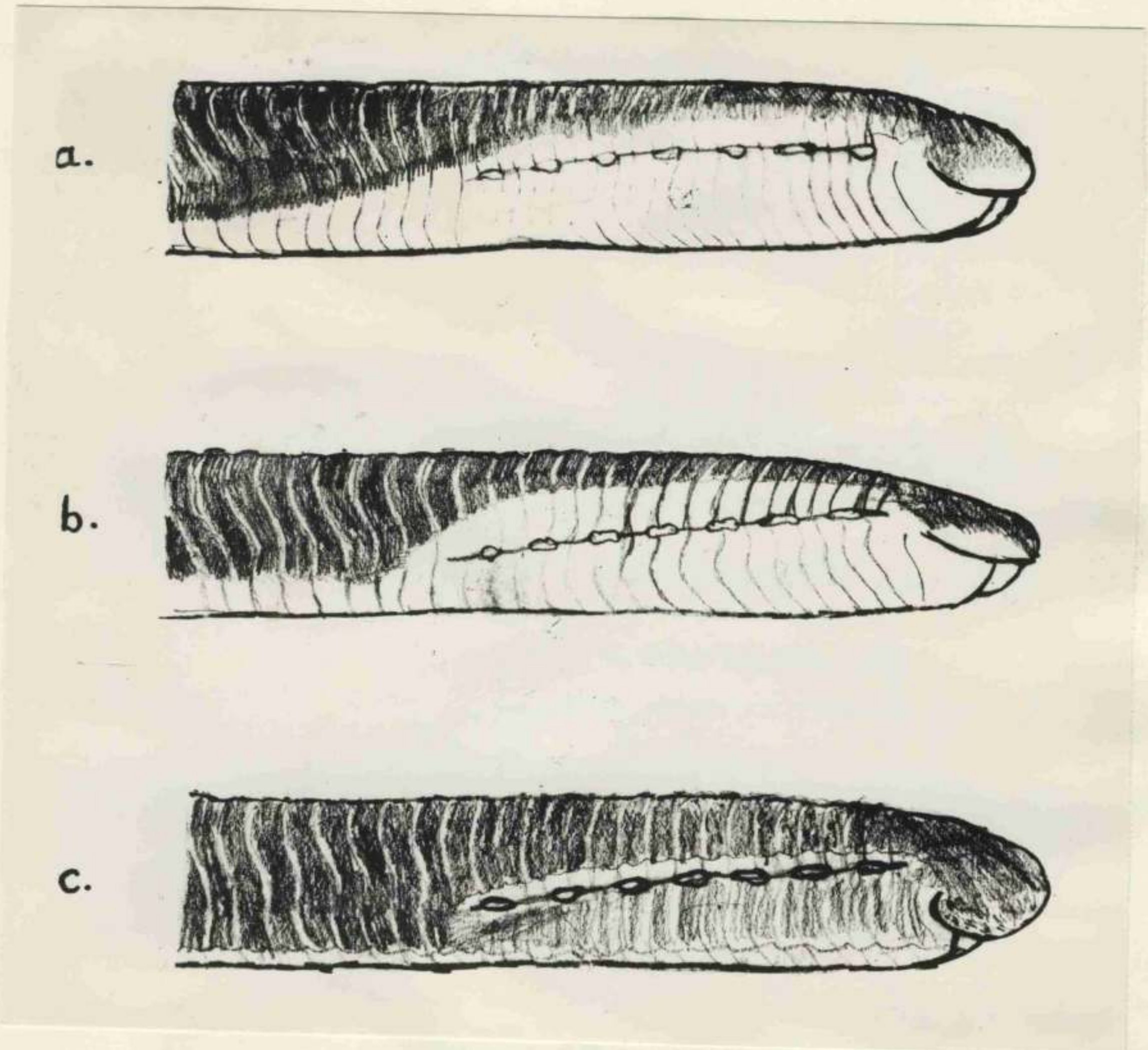


Fig. 6: Branchial regions of ammocoetes.
Magnification: 6X.
(a) L. planeri.
(b) L. fluviatilis.
(c) P. marinus.

of L. fluviatilis in this regard, so that this characteristic is good for segregating younger ammocoetes of L. planeri from those of the other two species. (See Figs. 4a, 4b, 4c).

(f) Shape of the Snout: L. fluviatilis ammocoetes possess a more pointed and beak-like snout than do L. planeri and P. marinus. In the latter two species the snout is blunt. This characteristic is suitable in comparing ammocoetes, but is not sufficiently distinctive to identify single specimens. (See Figs. 6a, 6b, 6c).

(g) Number of Trunk Myomeres: The number of trunk myomeres in ammocoetes is a very sound characteristic for segregating larvae of L. fluviatilis from those of L. planeri and P. marinus, when dealing with either living or dead material, as the myomeres can be seen very easily in ammocoetes. The following table presents the relevant data:

| Species | Range in No. Myomeres | Average No. Myomeres | Number Ammocoetes |
|---|---|---|---|
| L. planeri | 62 - 69 | 65.2 | 6,043 |
| L. fluviatilis | 51 - 58 | 54.4 | 4,102 |
| P. marinus | 61 - 69 | 67.2 | 1,184 |

The intrinsic value of myomere counts as a taxonomic criterion is that they can be made on the tiniest larvae, and remain constant throughout the whole life of the ammocoete. During metamorphosis the number of myomeres increases slightly, confusing the issue. But at this level of development identification is easily accomplished through the use of pigmentary, and other, characteristics.

THE PROBLEM OF RATIOS OF BODY PARTS:

In all of his taxonomic keys to species of adult lampreys, Vladykov (1949, 1955, 1958) has emphasized the importance of the relative sizes of certain body parts common in all lampreys as good criteria for separating species. The sections of the body used are the following:

(a) The distance from the tip of the disc to the first gill-slit. Abbreviated by - - - - - - - - - - - d - $B_1$.

(b) The distance from the 1st. to the 7th. gill - - - - - - - - - - - - - $B_1$ - $B_7$.

(c) Distance from 7th. gill-slit to anus - - - - - - - - - - - - - - - - - $B_7$ - a.

(d) Distance from anus to tip of caudal fin - - - - - - - - - - - - - - a - C.

(e) The length of longest fin ray in 2nd. dorsal fin - - - - - - - - - - - - - - - - - $hD_2$.

(f) Horizontal diameter of eye - - - - - - - O.

In view of the fact that Vladykov has never found these criteria to fail in segregating species of lampreys, the present author was most disappointed to find that no difference could be found among any of the British lampreys when compared on this basis. Fifteen adults of each species were measured. As the same techniques had not been tried on ammocoetes, ten L. planeri larvae, twelve L. fluviatilis and eleven P. marinus were examined with regard to this criterion. The results are shown in Tables II - IV and demonstrate quite clearly that, so far as British ammocoetes are concerned, this is an unreliable characteristic for species identification. The idea of expressing the figures as percentage ratios:

e.g. $\frac{d - B_1}{TL} \times 100$ is Vladykov's. TL means "total length".

TABLE II - SHOWING RELATIVE SIZES OF VARIOUS BODY PARTS OF AMMOCOETES OF *L. planeri* EXPRESSED AS PERCENTAGE RATIOS OF TOTAL LENGTH.

| App. Age (months) | TL (mm) | $\frac{d - B_1}{TL}$ | $\frac{B_1 - B_7}{TL}$ | $\frac{B_7 - a}{TL}$ | $\frac{a - C}{TL}$ | $\frac{hD_2}{TL}$ | No. of Specimens |
|---|---|---|---|---|---|---|---|
| 4 | 12 | 11.5 | 8.8 | 50.5 | 25.1 | 3.5 | 1 |
| 10 | 27 | 10.8 | 12.6 | 51.2 | 22.5 | 3.9 | 1 |
| 16 | 93 | 11.2 | 11.4 | 50.8 | 24.2 | 2.1 | 1 |
| 22 | 90 | 11.7 | 12.1 | 50.0 | 24.6 | 2.1 | 1 |
| 28 | 95 | 11.3 | 10.5 | 50.5 | 23.4 | 2.3 | 1 |
| 34 | 91 | 9.5 | 11.0 | 49.2 | 25.1 | 2.6 | 1 |
| 40 | 119 | 9.1 | 11.3 | 50.1 | 25.7 | 3.9 | 1 |
| 46 | 116 | 10.1 | 11.8 | 50.5 | 26.3 | 3.2 | 1 |
| 52 | 148 | 10.9 | 11.2 | 51.4 | 26.4 | 3.5 | 1 |
| 58 | 129 | 10.4 | 11.0 | 49.5 | 26.0 | 3.7 | 1 |
| MEANS: | 92.0 | 10.7 | 11.2 | 50.4 | 24.9 | 3.1 | 1 |

TABLE III - SHOWING RELATIVE SIZES OF VARIOUS BODY PARTS OF AMMOCOETES OF *L. fluviatilis* EXPRESSED AS PERCENTAGE RATIOS OF TOTAL LENGTH.

| App. Age (months) | TL (mm) | $\frac{d - B_1}{TL}$ | $\frac{B_1 - B_7}{TL}$ | $\frac{B_7 - a}{TL}$ | $\frac{a - C}{TL}$ | $\frac{hD_2}{TL}$ | No. of Specimens |
|---|---|---|---|---|---|---|---|
| 4 | 30 | 9.1 | 11.4 | 49.7 | 26.8 | 3.6 | 1 |
| 10 | 35 | 9.8 | 10.2 | 52.1 | 26.4 | 2.3 | 1 |
| 16 | 73 | 11.5 | 11.9 | 51.2 | 25.4 | 3.0 | 1 |
| 22 | 79 | 11.6 | 10.6 | 52.0 | 27.1 | 3.9 | 1 |
| 28 | 94 | 10.1 | 11.0 | 50.7 | 25.6 | 3.7 | 1 |
| 34 | 110 | 11.1 | 10.6 | 50.5 | 24.9 | 1.1 | 1 |
| 40 | 115 | 11.8 | 11.4 | 50.0 | 25.8 | 3.5 | 1 |
| 46 | 131 | 11.3 | 9.1 | 50.3 | 25.0 | 3.9 | 1 |
| 52 | 152 | 9.1 | 11.2 | 49.2 | 26.5 | 3.5 | 1 |
| 58 | 140 | 11.4 | 10.5 | 51.7 | 25.1 | 3.3 | 1 |
| 64 | 195 | 11.7 | 9.3 | 50.1 | 25.4 | 3.0 | 1 |
| 70 | 144 | 11.0 | 11.2 | 51.0 | 23.9 | 3.2 | 1 |
| MEANS: | 108.2 | 10.8 | 10.7 | 50.8 | 25.6 | 3.2 | 1 |

TABLE IV - SHOWING RELATIVE SIZES OF VARIOUS BODY PARTS OF AMMOCOETES OF *P. marinus* EXPRESSED AS PERCENTAGE RATIOS OF TOTAL LENGTH.

| App. Age (months) | TL (mm) | $\frac{d - B_1}{TL}$ | $\frac{B_1 - B_7}{TL}$ | $\frac{B_7 - a}{TL}$ | $\frac{a - C}{TL}$ | $\frac{hD_2}{TL}$ | No. of Specimens |
|---|---|---|---|---|---|---|---|
| 4 | 19 | 11.3 | 11.2 | 51.6 | 24.9 | 2.1 | 1 |
| 10 | 30 | 11.4 | 12.7 | 47.5 | 27.8 | 2.1 | 1 |
| 16 | 82 | 11.3 | 10.0 | 50.3 | 25.7 | 2.4 | 1 |
| 22 | 80 | 11.7 | 10.6 | 50.1 | 25.0 | 2.9 | 1 |
| 28 | 111 | 11.8 | 9.2 | 50.9 | 24.3 | 3.1 | 1 |
| 34 | 130 | 10.6 | 10.2 | 49.8 | 24.2 | 4.9 | 1 |
| 40 | 140 | 11.0 | 10.5 | 50.6 | 26.9 | 2.1 | 1 |
| 46 | 160 | 11.5 | 10.1 | 52.3 | 24.7 | 3.8 | 1 |
| 52 | 189 | 9.8 | 11.9 | 50.7 | 24.2 | 3.1 | 1 |
| 58 | 196 | 11.0 | 11.5 | 50.6 | 25.6 | 2.0 | 1 |
| 64 | 240 | 11.4 | 11.5 | 46.4 | 24.4 | 3.1 | 1 |
| MEANS: | 125.2 | 12.1 | 10.8 | 50.1 | 25.2 | 2.9 | 1 |

FACTORS CAUSING VARIATION IN PIGMENTATION:

There are, in an ammocoete's environment, in-:numerable variables which might be expected to reflect themselves in the intensity or distribution of external pigment in the ammocoete. However, after collecting ammocoetes widely throughout Britain, at various times of year and by various means, and after having had several years' experience in maintaining ammocoetes in the labor-:atory, the author has come to the conclusion that only two factors cause sufficient variation in pigmentation to be of merit in this paper.

(a) Seasonal Fluctuations in Temperature: Extreme caution must be exercised in examining en masse ammo-:coetes which have been taken at different times of year. It was found, for instance, that ammocoetes of L. planeri taken during the winter months were far darker in colour than those taken from the same river (Endrick) in summer. This difference was so marked that the summer ones were almost misidentified. The portion of the body most strongly affected by temperature fluctuations is the branchial region, while the caudal fin is scarcely affected at all. The tendency toward a fading of external

a

b

Fig. 7: Variations in intensity of pigmentation on the branchial region of L. planeri ammocoetes taken from the River Endrick in Scotland. Magnification: 3X.
(a) larva taken in mid-July.
(b) larva taken in mid-February.

pigments in the summer and an intensifying of them in the winter is more readily seen in L. planeri than in the other species (Fig. 7).

(b) Regional Distribution: In comparing ammocoetes caught under similar seasonal conditions on the east and west coasts of Britain, the conclusion was reached that East-West distribution has no significant effect on the intensity of pigmentation, but that some variation does occur on a North-South axis. (Fig. 8) shows that ammo:coetes collected in Scottish streams in summer tend to be quite noticeably darker than ammocoetes collected at nearly the same time of year in southern England. Again this difference is most distinct in ammocoetes of L. planeri.

SUMMARY: Although no one of the characteristics (with the possible exception of those dealing with the pre:cursor of the tongue) described is dependable for separating all three species of ammocoetes, they can be combined in a number of ways, according to the condition of the material, whether it must be identified alive, etc., to produce several valid keys. The following one is recommended.

a

b

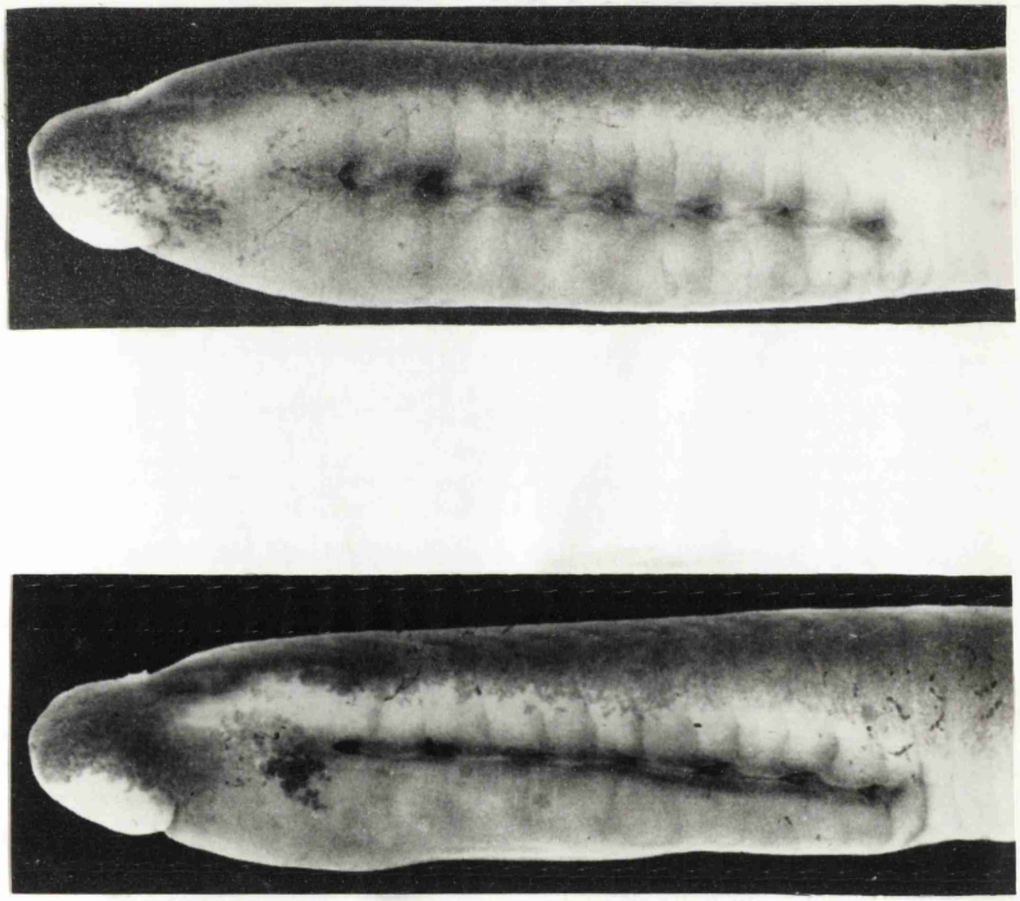

Fig. 8: Variations in intensity of pigmentation on the branchial region of L. planeri ammocoetes taken in July. Magnification: 3X.
(a) from the Isis River, Oxford, England.
(b) from the River Leven, Scotland.

A KEY TO AMMOCOETES OF BRITISH LAMPREYS:

$A_1$ - A maximum of 58 Trunk Myomeres - - L. fluviatilis.

$A_2$ - A minimum of 60 Trunk Myomeres.

- $B_1$ - Area of Caudal Fin largely of a milky transparancy, with many small dark melanophores in a narrow margin outlining the posterior tip of the body - - - - - - - - - - - - - - L. planeri.

- $B_2$ - Area of Caudal Fin sprinkled widely with grey melanophores P. marinus.

The above key would be of use in segregating living ammocoetes. The specimen would have to be placed in a test-tube of sufficient narrowness to prevent it from writhing too violently, and the myomere counts made with a magnifying lens.

It should be stressed, however, that if dead material is being examined with a view to identification, all of the criteria should be called into play.

## EARLY LARVAL DEVELOPMENT:

### SPAWNING:

As a prerequisite to studying the rate of development of ammocoete embryos so as to obtain complete data on length of larval life, certain observations were made on spawning adults. This was done in order that the eggs could be studied immediately after fertilization, and that the conditions of water temperature, etc., re-:quired by the early larval stages could be assessed directly.

Several exhaustive studies have been made on the spawning behaviour of adult lampreys, especially the three species concerned in this paper. Lilljeborg (1891), Smitt (1895), Weissenberg (1925), Lauterborn (1926), Cotronei (1927), Ivanovaberg (1936), Clare (1939), Bahr (1952) and Hagelin and Steffner (1958) all investigated Lampetra fluviatilis from this point of view, so that my own work on this problem is merely a superficial check to ascertain whether British River Lampreys engage in spawning behaviour noticeably different from that observed on the continent.

Spawning habits of various types of small Brook Lampreys, all very similar to Lampetra planeri, have also been very competently investigated by a number of workers, as follows: Lampetra* wilderi: Dean, Eastman and Sumner (1898), Gage (1896), Young and Cole (1900), Reighard (1903). Lampetra planeri: Muller (1856), Loman (1912), Schultz (1930), Hardisty (1944). Ichthyomyzon greeleyi: Hubbs and Trautman (1937). Hardisty was the only one to carry out his work in Britain.

Spawning behaviour in the Sea Lamprey has only been fully investigated in the United States; Hussakof (1912), Coventry (1922) and Applegate (1950).

Comparison of these researches show that there is very close agreement about the optimum conditions for spawning lampreys of all species:

(a) Spawning is carried out over sandy pebbled substrates.

(b) Whenever possible lampreys seek out shaded areas of the stream for spawning.

*Until 1900 the various American types of Brook Lampreys were presented under the generic name of PETROMYZON.

(c) The eggs are fertilized at or near a water temperature of 11°C.

Lamsa (1956) showed that the spawning runs of Sea Lampreys take place at night.

In view of the absence of any comprehensive investigation of spawning Sea Lampreys in Europe, it is indeed unfortunate that the present author was unable to undertake such a study. However, three attempts to maintain living Sea Lampreys in tanks failed, and field studies on this animal were largely confounded by the scarcity and inaccessibility of its spawning grounds.

Spawning of River Lampreys (L. fluviatilis) and of Brook Lampreys (L. planeri) was investigated both in the laboratory and in the field. Baxter (1949) made a number of good suggestions concerning the maintenance of adult lampreys in captivity. In making laboratory observations these were followed as far as possible.

Lampetra planeri: Early in February 1958, 9 adult Brook Lampreys were taken from the Field Station Burn* and were

*A small stream running from a spring near the Lodge House of the Rossdhu estate (which lies between Balloch and Luss) for a few hundred feet into the west side of Loch Lomond.

kept in an aquarium under as natural conditions as possible. As well, observations were made regularly on the Field Station Burn itself, and the Inler Burn*. Daily records were kept of water temperature, both in the aquarium and in the field.

Beginning on 19 April, 1958, an all day vigil, from dawn until sunset, was made every third day at the laboratory, the Inler Burn and the Field Station Burn respectively. This was done in the hope that L. planeri would spawn under daylight conditions when they could be observed. This hope was justified, and observations were continued in this manner until 7 May, 1958, by which time the laboratory stock of L. planeri had died off, and spawning of this species appeared to be at an end in both the Inler and Field Station Burns.

Exact counts of the numbers of Brook Lampreys constituting each spawning run in the field could not be made due to the poor visibility in both burns, but particularly the Inler, which is used as a sewage ditch.

*A tributary burn to the River Leven, Danbartonshire, about 1km. from Loch Lomond.

Data derived from these experiments are to be found in Table V, where the letter "s" signifies that spawning was seen on that day, while the letter "n" signifies that either no lampreys were seen (which, of course, could never be the case where laboratory stock was involved), or that those which were seen were not spawning. As stated earlier, observation could only be made on every third day in each of the three places. Dashes signify that no observations were made.

The fact that L. planeri were seen spawning during the day does not preclude the possibility that they also spawn at night, nor is it an indication that light has any control over spawning, as in both burns spawning was carried out only in the shaded areas of the stream.

Lampetra fluviatilis: As with the Brook Lampreys, River Lampreys were observed spawning both in the field and in the laboratory. Field observations on this species were also made in the Field Station Burn and the Inler Burn.

At the beginning of January 1958, twenty-three adult River Lampreys were trapped in the Severn River near Tirley, Gloucestershire. Of these, seven survived

TABLE V - SPAWNING OF *L. planeri* IN TWO STREAMS AND IN THE LABORATORY.

| | Field Station Burn | | Inler Burn | | Laboratory | |
|---|---|---|---|---|---|---|
| Date | s/n | Mean Water Temp. ($^{o}$C) | s/n | Mean Water Temp. ($^{o}$C) | s/n | Mean Water Temp. ($^{o}$C) |
| Apr. 21 | - | - | - | - | n | 10.5 |
| Apr. 22 | - | - | n | 10.0 | - | - |
| Apr. 23 | n | 10.5 | - | - | - | - |
| Apr. 24 | - | - | - | - | n | 11.5 |
| Apr. 25 | - | - | n | 11.0 | - | - |
| Apr. 26 | s | 11.0 | - | - | - | - |
| Apr. 27 | - | - | - | - | s | 11.5 |
| Apr. 28 | - | - | s | 11.0 | - | - |
| Apr. 29 | s | 11.5 | - | - | - | - |
| Apr. 30 | - | - | - | - | s | 11.5 |
| May 1 | - | - | s | 11.5 | - | - |
| May 2 | n | 12.5 | - | - | - | - |
| May 3 | - | - | - | - | n* | 12.5 |
| May 4 | - | - | n | 12.5 | - | - |
| May 5 | n | 12.0 | - | - | - | - |
| May 6 | - | - | - | - | n | 13.0 |
| May 7 | - | - | n | 12.5 | - | - |

*By this time all of the laboratory stock of *L. planeri* had died. Dissection revealed that they had all spawned.

the winter in the laboratory, and six of them spawned successfully.

Unfortunately, it quite quickly became apparent that River Lampreys are only active after dark. In the laboratory attempts were made to watch them at night by use of a dimmed electric torch. However, even this dis:turbed them, causing them to cease moving almost immedi:ately. Those seen during the day time in the Inler Burn were invariably at rest, holding onto a rock or some other surface with their sucking discs. This, of course, eliminated the possibility of studying their spawning habits in the field. However, in the laboratory it was possible to determine roughly what they were doing at night by allowing about half an hour for the eyes to accommodate to darkness.

On the morning of 2 May, 1958, eggs were found in the nest, indicating that spawning had taken place some time the night before. No observations could be made on 3rd. or 4th. May, but on the morning of the 5th. it was discovered that more eggs had been shed in the interim. On May 7th. six of the seven lampreys died, and dissection of them revealed that they had spawned. The last one, a female, did not die until several days later, at which time dissection showed that although sexually mature, it had not spawned.

Petromyzon marinus: It has been shown by field experiments in which Sea Lamprey were trapped as they made their way upstream (Lamsa 1956) that they were most active between 10 p.m. and 1 a.m. and at a water temperature of about 14°C (Scott 1956).

On May 4th., during the day, a Sea Lamprey nest with several adults nearby was found in the River Fruin. In the nest were found several hundred eggs. Neither the lampreys nor the eggs had been there on the 3rd. The water temperature was 12°C.

In considering this spawning information, then, one comes to the conclusion either that L. planeri and L. fluviatilis eggs can only successfully begin development at a temperature not lower than 11°C. and not higher than 12°C. at the most, or that it is the time of year which determines when the eggs are shed. Hardisty (1944) found that, in L. planeri, a water temperature of between 10°C. to 11°C. was optimum for spawning, and found that in the river system with which he was dealing, spawning took place in late March or early April.

Too much weight cannot be placed on the rather high water temperature recorded for spawning Sea Lampreys in Canada (Scott 1956), because the streams concerned are subjected to wide fluctuations of temperature in the Spring and early Summer. Even so, the fact that such discrepan-:cies in optimum spawning temperature have been recorded, underlines the need for further research in this field.

EMBRYOLOGY:

Scott (1888) made a noteworthy attempt to study the embryology of River Lampreys (L. fluviatilis), but it is quite evident that he was handicapped by his tech-:niques.

Approximately 200 fertilized eggs of L. planeri and the same number of L. fluviatilis eggs were transferred from the aquaria in which the spawning had taken place to two Kilner jars half filled with water, L. fluviatilis eggs in one and L. planeri eggs in the other. Mosquito netting was then secured over the open mouths of the jars, and each was kept lying on its side in a stream of con-:stantly running water in one of the aquaria. Fertilized eggs of P. marinus obtained from a nest in the Fruin River were treated likewise.

For the first 10 hours, a few eggs of both L. planeri and L. fluviatilis were examined hourly. This could not be done with the P. marinus eggs, because no way of knowing exactly when they had been shed was available. After this a few eggs of all three species were examined at the end of each day, until the larvae had developed sufficiently to become motile.

When this stage had been reached, a sample of ten larvae of each species was killed and measurements of length were taken. The results are shown in Table VI. The ten larvae of each species all became motile within a few hours of one another. The greatest time spread was found in P. marinus in which the first motile larva was noted on 25 May, 1958 at 10:35 a.m., while the last to become motile did not do so until 2:20 p.m. on the same day.

In all three species the cleavage of the egg was holoblastic (See Fig. 9). It is worthwhile noting the differences in time required by L. fluviatilis and L. planeri to attain motility. This would seem to strengthen further the present author's view that these can be regarded as separate species. This has been a point of debate among British systematists for some years.

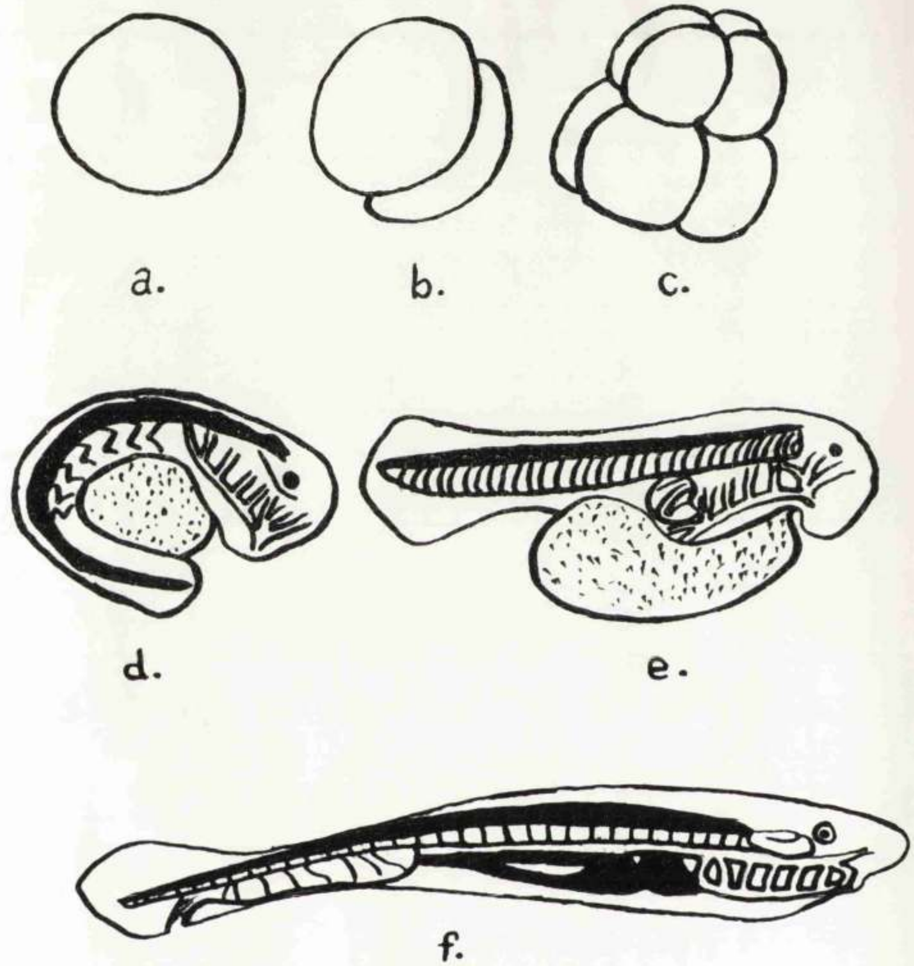


Fig. 9: Embryological development in L. planeri.
Magnification: 25X.
(a) Fertilized egg (d) 15th. day
(b) First Cleavage (5 hours) (e) 16th. day
(c) 8 cell stage (8 hours) (f) 19th. day

TABLE VI - DEVELOPMENT OF EGGS OF L. planeri, L. fluviatilis AND P. marinus IN LABORATORY UNTIL LARVAE ARE MOTILE.

| Species | Date Fertilized | Date Larvae Became Motile | Average Length at Motility | Age in Days at Motility |
|---|---|---|---|---|
| L. planeri | Apr. 30 | May 22 | 7.7 mm. | 22 days |
| L. fluviatilis | May 5 | May 19 | 5.2 mm. | 14 days |
| P. marinus | May 3 or 4 | May 27 | 5.4 mm. | app. 23 days |

RATE OF GROWTH OF MOTILE LARVAE TO 15 - 20 MILLIMETRES IN LENGTH:

In the jars with the newly motile larvae a thin layer of sand was placed so that the ammocoetes could take up as natural an existence as possible without be-:coming too difficult to find for future examination. Every week, two larvae of each species were removed from their respective jars, killed and measured. It was in-:tended that, if a difference of greater than 0.5 milli-:metres was recorded between the length of the two speci-:mens of any single sample, a third larva of the same species would be measured and the three values averaged. In point of fact this never had to be done, indicating that at that stage of their development, at least, lamprey larvae grow at a very uniform rate.

Results of this experiment are shown in Table VII. As a glance at these figures will reveal, the rate of linear growth for approximately the first 16 weeks is only indicated roughly. This was due to the small samples used, no doubt. For instance, between the dates 25 August and 8 September Sea Lamprey ammocoetes are recorded as having lost 0.4 millimetres length and then regained it. This is more likely the result of sampling error than of any intrinsic physiological development within the ammocoete. However, the figures are sufficiently accurate to show that rate of growth at this level is very slow.

TABLE VII - AVERAGE RATES OF GROWTH OF AMMOCOETES THROUGH FIRST 16 WEEKS OF ACTIVE LIFE.

| | Length in Millimetres of all Three Species | | |
|---|---|---|---|
| Date | L. planeri | L. fluviatilis | P. marinus |
| May 26 | 7.9 | 5.1 | 5.7 |
| Jun 2 | 8.1 | 5.8 | 5.8 |
| Jun 9 | 8.4 | 6.1 | 6.6 |
| Jun 16 | 8.7 | 6.5 | 7.4 |
| Jun 23 | 9.2 | 6.8 | 8.1 |
| Jun 30 | 9.9 | 7.2 | 8.7 |
| Jul 7 | 10.4 | 7.6 | 9.4 |
| Jul 14 | 11.4 | 8.3 | 11.2 |
| Jul 21 | 11.8 | 9.0 | 11.1 |
| Jul 28 | 12.8 | 10.1 | 11.2 |
| Aug 4 | 13.5 | 12.0 | 12.6 |
| Aug 11 | 14.7 | 15.0 | 12.9 |
| Aug 18 | 15.4 | 15.4 | 13.6 |
| Aug 25 | 16.9 | 16.3 | 14.4 |
| Sep 1 | 17.5 | 17.1 | 14.0 |
| Sep 8 | 18.8 | 19.7 | 14.5 |
| Sep 15 | 17.8 | 21.6 | 15.1 |

ESTIMATES OF RATES OF LINEAR GROWTH IN AMMOCOETES:

Long before ammocoetes were studied from the point of view of year classes, several important investi-:gations on the sequence of developments, mainly anatomi-:cal, which manifest themselves during ammocoete growth and metamorphosis, were carried out.

Outstanding in this regard is an extremely thorough anatomical study made by Daniel (1931) on two types of Brook Lampreys found in the United States. Leach (1940) made a similar but more superficial analysis on another American Brook Lamprey, relating his data to the local ecology of the species.

Although no attempt was made by either of these authors to establish year classes, their papers are valu-:able in that landmarks of ammocoete development are des-:cribed and placed in their correct sequence, so that future age group analysts were provided with a dependable method of verifying their conclusions.

The importance of this contribution is best seen by considering attempts at age group analysis made pre-:vious to the work of Daniel and Leach. Hubbs (1924), who attempted to ascertain the length of larval life in

Brook Lampreys, made the now classical error of regarding the largest ammocoetes as the oldest, being totally un-:aware that a marked decrease in length precedes metamor-:phosis.

Okkelberg (1922) described the life cycle of the Brook Lamprey (Ichthyomyzon unicolor) without attempting to segregate year classes.

An understandably more intriguing aspect of growth study in ammocoetes is the problem of metamorphosis and the various profound changes associated with it. In both Europe and America this has captured the imagination of a number of eminent biologists, but two are of particu-:lar importance from the point of view of this paper.

Gage (1898), investigating metamorphosis in Lampetra wilderi, noted the various stages through which the larvae pass, in particular, the progressive develop-:ment of the mouth anatomy is described in detail. Balabai (1948) dealing with a European Brook Lamprey (Lampetra mariae), ascertained that the length of time required for metamorphosis lies in the region of four to five months.

As has already been pointed out, all previous attempts to ascertain rates of growth in ammocoetes have in reality been only estimates based on random sampling. Also only the larvae of the Brook Lamprey (Lampetra sp.) have been considered from this point of view, the other two British species having been totally ignored.

Even though only one species of ammocoete has been dealt with in these previous experiments, the con-:clusions to which the various investigators have come have not agreed, despite the fact that all used essen-:tially similar methods. Schultz (1930) and Hardisty (1944) estimated a larval life of between three and four years for ammocoetes of L. planeri, while Knowles (1941) estimates a larval life of four to five years for the same type. Ivanova-Berg (1931) came to the conclusion that larval life lasts five years. Hardisty (1951) re-assessed his work, using larger samples, and reached the conclusion that the total life span, including adulthood, of L. planeri lasts for not more than six years, and that the ammocoete stage probably lasts for just over five years. He avers that there is some variation in the length of larval life according to stream conditions from one year to the next, substrate type, etc. This claim is sub-:jected to experimental analysis later on in this paper.

Since at the time that these experiments were carried out, no method of classifying British ammocoetes had been developed, it seems reasonable to conclude that perhaps one or more of the investigators concerned had taken mixed samples of ammocoetes, thereby confusing size class analysis.

In the light, then, of these considerations, and in view of the fact that a system for selectively identi:fying species of ammocoetes has now been developed (Pp. 34) it was thought advisable to make further esti:mates of rates of linear growth in ammocoetes by the sampling methods employed by previous investigators, and including all three species in the survey, before carrying out laboratory experiments on growth under control con:ditions. As a preliminary to such an analysis search was conducted in various rivers to obtain pure samples of each species. Although the methods of identifying larval lampreys are quite dependable for larger specimens, they are not such satisfactory criteria when dealing with small ammocoetes. Therefore, all mixed samples were rejected.

Finally three pure samples of each species were obtained, each sample being taken at a different time of year. All ammocoetes thus taken were preserved in 4%

formalin solution on the spot. Later they were transferred to 70% preserving alcohol. Measurements were always taken within a day of capture. Every ammocoete measured was also weighed and measurements were taken of certain sections of the body. These latter two factors will be discussed later.

Also, in sampling, care was taken to collect ammocoetes systematically across the river or stream concerned, as the author had reason to believe that certain age-groups of ammocoetes favour different depths of water and types of substrate (MacDonald 1956).

Lampetra planeri:

All three samples of L. planeri ammocoetes were taken from the Field Station Burn. It was thought at the time that this analysis was begun that such a source would be sure to yield only Brook Lamprey larvae because the shallowness of the stream is such that one would suppose that larger lampreys could not enter from Loch Lomond. Also, since it is so convenient to the University Field Station, spawning Brook Lampreys and no other species of lampreys had been observed in it many times. As events turned out, it was later discovered that River Lampreys ascend this burn at night during the spring spawning runs. However, the ammocoete key was sufficiently developed by this time to ascertain which larvae were those of L. planeri, and which were those of L. fluviatilis. The figure in parenthesis indicates the number of specimens of that particular size class taken.

Sample A: Taken by dredging on December 3, 1957.
36, 37, 38, 41, 67, 118, 129, 130, 133, 134, 135, 139, 140(2), 141, 143, 148, 149, 152, 153, 170, 171, 173. Total: 23.

The three largest were in advanced stages of metamorphosis, the lateral eyes being open and "teeth" having been formed in the oral cavity. However, the sucking disc was not sufficiently developed for the ammo-:coetes to anchor themselves with it.

Sample B: Taken by electric shocker and by dredging on February 19, 1958.
34, 35, 36, 37, 71, 72, 75, 76, 77, 78, 79, 80, 81, 118, 119, 120, 122, 123, 136, 137, 141, 142, 143, 144, 147, 151, 152, 153, 154, 156, 158, 159, 148,* 149.*

The total number of ammocoetes taken was 34, and two of them (148 and 149 millimetres long respectively) had completed metamorphosis in that they had developed a functional sucker, and as well were armed with the proper compliment of "teeth" and had both lateral eyes open. However, they were still burrowed in the mud, indicating that they were not quite ready for adult life.

Sample C: Taken by electric shocker and by dredging July 30, 1958.

15(5), 16(3), 17(2), 18(6), 19(4), 20, 21, 53, 57, 64, 67, 74, 78, 80, 81(2), 101(2), 103(4), 116, 118, 120, 125, 128, 129, 132, 136, 140, 168. Total: 50 ammocoetes.

The largest specimen had partly opened lateral eyes, indicating that metamorphosis had commenced.

Analysis of any of these samples shows that they fall into five well defined size classes, without overlap. The data are shown in this form in Table VIII. The samples are listed in order of their occurrence after spring spawning, not in the chronological order in which they were taken. This was done in order to facilitate interpretation of the data.

TABLE VIII - SIZE CLASSES DERIVED FROM THREE SAMPLES OF AMMOCOETES OF *L. planeri*.

| Sample | Date Taken | Size Class | Range in Length (mm) | Average Length (mm) | Number of Specimens |
|---|---|---|---|---|---|
| C | Jul 30/58 | 1 | 15 - 21 | 17.5 | 23 |
| | | 2 | 50 - 81 | 69.1 | 11 |
| | | 3 | 101 -103 | 102.3 | 6 |
| | | 4 | 116 -140 | 125.3 | 9 |
| | | 5 | 168 - | 168.0 | 1 |
| | | | | | 50 |
| A | Dec 3/57 | 1 | 36 - 41 | 38.0 | 4 |
| | | 2 | 67 - | 67.0 | 1 |
| | | 3 | 118 - | 118.0 | 1 |
| | | 4 | 129 -153 | 140.4 | 14 |
| | | 5 | 170 -173 | 171.3 | 3 |
| | | | | | 23 |
| B | Feb 19/58 | 1 | 34 - 37 | 35.5 | 4 |
| | | 2 | 71 - 81 | 79.6 | 9 |
| | | 3 | 118 -123 | 120.4 | 5 |
| | | 4 | 136 -159 | 148.1 | 14 |
| | | 5 | 148 -149 | 148.5 | 2 |
| | | | | | 34 |

Considering Sample A of Table VIII, since it has been demonstrated that Brook Lampreys spawn about April, it is fair to assume that the ammocoetes of Size Class 1 hatched a matter of three or four months previously, that Size Class 2 ammocoetes were a year older, Size Class 3 ammocoetes 2 years older, etc. The one ammocoete constituting Size Class 5 was thus probably 4 years older than the Size Class 1 ammocoetes, and so could be considered to be in its fifth year of larval life.

This, then, would indicate that the larval life of L. planeri lasts over four years and less than 5 years. Consideration of Sample B lends strength to this view, because in the four month interim separating it from Sample A, the ammocoetes of Size Class 1 would have had time to grow an average of 20.5 millimetres in length (from 17.5 mm. to 38.0 mm.), and so on through the other size classes.

Comparison of Samples B and C gives the impression that little, if any linear growth takes place in larvae of L. planeri during the months of December, January and February. This hypothesis is examined more critically under laboratory conditions elsewhere in this paper (see Pg. 68). In considering Sample C alone, it is seen that two of the ammocoetes had metamorphosed, but had not yet discarded the burrowing habit. Both of these ammocoetes are smaller, on the average, than Size Class 5 ammocoetes of Sample B, and even of Sample A.

It has been known for some time that newly metamorphosed Brook Lamprey are somewhat smaller than certain bigger ammocoetes of the same species. Vladykov (1949) gives the average length of newly metamorphosed Brook Lamprey as being 143.0 millimetres for females, and 138.5 millimetres for males. Both of these figures are, of course, mean values.

The present author has found that, in a sample in which both sexes were included without distinction, the average length of L. planeri adults in different stages of sexual maturity is 144.7 millimetres. It has been hypothesized by several investigators (Hardisty 1941, Schultz 1930, etc.) that Brook Lamprey do not feed during metamorphosis and adult life. If this is the case, it seems reasonable to assume that some shrinkage takes place during this period of starvation.

Lampetra fluviatilis:

The three pure samples of River Lamprey ammo-:coetes were taken from the Fruin River, and, as was the case with L. planeri larvae, the first sample was collected by means of dredge net alone, while the other two were taken with the aid of both electric shocker and dredge nets.

Sample A: Taken on November 5, 1957.
23(2), 24, 27, 28, 31, 58, 60, 61(4), 65, 87, 88, 89, 128, 129, 130, 132, 133, 158, 160, 162, 195, 196(2), 198(2).

A total of 29 ammocoetes were taken. The five largest animals were in the advanced stages of metamor-:phosis, as was indicated by the lateral eyes being open, "teeth" being present in the oral cavity, etc.

Sample B: Taken on February 23, 1958.
24, 25(3), 27, 29(2), 53(2), 54, 55, 58(2), 59(5), 86, 130, 131, 157, 158, 160(5), 171(2), 172, 175, 176.

Two of these ammocoetes (130 and 131 millimetres in length respectively) had metamorphosed, but were still burrowed in the mud.

Sample C: Taken on June 20, 1958.

11, 13, 14, 43, 44, 46, 47(3), 48, 51, 52, 54, 55, 56(2), 57, 58, 60, 61, 62, 63, 67(5), 69, 80, 82, 83, 84(3), 85, 87(3), 113, 114(2), 116, 118, 119(2), 120, 149, 151, 152(2), 153, 157, 158(3), 185, 186, 188.

All of these samples, except the second (B), fell without overlap into several well defined size classes. In Sample B, the two metamorphosed ammocoetes were regarded as constituting a separate size class, representing the final year of larval life.

are/ The data ~~is~~ presented in Table IX, where the samples are listed in order of their occurrence after spring spawning rather than in the true order in which they were taken.

From Table IX, it is seen that Size Class 1 ammocoetes of Sample A probably hatched earlier in the same summer. Size Class 2 ammocoetes can then be considered a year older, and so forth. By November 5th., assuming no significant difference in rate of growth between the summers of 1958 and 1957, an average of 13.3 millimetres increase in length has taken place in Size Class 1 ammocoetes, and so on down the line.

TABLE IX - SIZE CLASSES DERIVED FROM THREE SAMPLES OF AMMOCOETES OF *L. fluviatilis*.

| Sample | Date Taken | Size Class | Range in Length (mm) | Average Length (mm) | Number of Specimens |
|---|---|---|---|---|---|
| C | Jun 20/58 | 1 | 11 - 14 | 12.7 | 3 |
| | | 2 | 43 - 69 | 56.4 | 25 |
| | | 3 | 80 - 87 | 84.3 | 10 |
| | | 4 | 113 - 120 | 116.6 | 8 |
| | | 5 | 149 - 158 | 154.2 | 9 |
| | | 6 | 185 - 188 | 186.3 | 3 |
| | | | | | 58 |
| A | Nov 5/57 | 1 | 23 - 31 | 26.0 | 6 |
| | | 2 | 58 - 65 | 61.0 | 7 |
| | | 3 | 87 - 89 | 88.0 | 3 |
| | | 4 | 128 - 133 | 130.4 | 5 |
| | | 5 | 158 - 162 | 160.0 | 3 |
| | | 6 | 195 - 198 | 196.5 | 5 |
| | | | | | 29 |
| B | Feb 23/58 | 1 | 24 - 29 | 26.3 | 7 |
| | | 2 | 53 - 59 | 56.9 | 11 |
| | | 3 | 86 - | 86.0 | 1 |
| | | 4 | 157 - 160 | 159.3 | 7 |
| | | 5 | 171 - 176 | 173.0 | 5 |
| | | 6 | 130 - 131 | 130.5 | 2 |
| | | | | | 33 |

Again, by comparing Samples B and C, one comes to the conclusion that no significant growth occurs in lamprey larvae during the winter months, and in Size Class 6 of Sample C (e.g. those ammocoetes which had metamorphosed) a significant decrease in body length (from an average of 196.5 to an average of 172.6 milli:metres) is evident.

From this data, then, one must arrive at the conclusion that the larval life of Lampetra fluviatilis lasts for slightly less than six years.

Petromyzon marinus:

Ammocoetes of Sea Lamprey were far more scarce in the areas searched by the present author than were larvae of either of the other two species. Finally, however, three pure samples were obtained, all having been taken from the Inler Burn (see Pp. 18). The first sample was collected with the hand dredge net, while in taking the subsequent two samples, the electric shocker was also employed.

Sample A: Taken on December 1, 1957.

34, 35(4), 36, 38, 44, 47, 48(2), 50, 85, 91, 97, 142, 146, 147, 186, 191, 194(2), 195, 235, 242, 248, 251, 223*, 228*, 231*(2), 232*, 238*, 239. Total: 34 ammocoetes.

Seven ammocoetes in this sample had completed metamorphosis, namely those designated with asterisks. All of these seven were in possession of "teeth", well developed lateral eyes, and functional sucking discs. The typical ammocoete colour had been replaced on two of them (228 and 239 millimetres in length) by the black dorsal surface and silvery ventral surface of the adult lamprey. However, they were still burrowed.

Sample B: Taken on March 15, 1958.

40(5), 41, 42(2), 43, 90, 91, 92, 93, 95, 148, 149, 190, 192, 193, 194(2), 244, 246. Total: 23 specimens.

Sample C: Taken on August 2, 1958.

23, 28, 30, 38, 68, 70, 71, 75, 83, 112, 119, 122, 125, 130, 141, 142, 146, 150, 155, 197, 202, 250, 253, 260. Total: 25 ammocoetes.

The three largest ammocoetes in this last sample were commencing metamorphosis, as was manifested by partly opened lateral eyes.

From the point of view of analysis, this data is rather more vague than that which was obtained from samples of River and Brook Lamprey ammocoetes. In Table X, the data is presented in the usual form.

In Sample A (December 1, 1957) the seven meta:morphosing ammocoetes should be considered as a separate Size Class. Sample B is straightforward enough, indicating that Sea Lampreys have a larval life of between five and six years. During the five month gap between Sample C and Sample A (assuming again that rates of growth in ammo:coetes are roughly the same each summer), considerable growth apparently takes place, except in Size Class 6 animals, which seem to suffer a significant decrease in length, if these samples are taken as representative. Since these ammocoetes were already metamorphosing, it is probably safe to associate this shrinkage with the effects of starvation, as metamorphosing ammocoetes do not feed.

In this connection it is interesting to note that in ammocoetes of <u>L. planeri</u> and <u>L. fluviatilis</u> metamorphosis commences in early Autumn and is not

TABLE X - SIZE CLASSES DERIVED FROM THREE SAMPLES OF AMMOCOETES OF P. marinus.

| Sample | Date Taken | Size Class | Range in Length (mm) | Average Length (mm) | Number of Specimens |
|---|---|---|---|---|---|
| C | Aug 2/58 | 1 | 23 - 38 | 15.3 | 4 |
| | | 2 | 68 - 83 | 73.4 | 5 |
| | | 3 | 112 - 130 | 121.6 | 5 |
| | | 4 | 141 - 155 | 146.8 | 5 |
| | | 5 | 197 - 202 | 199.5 | 2 |
| | | 6 | 250 - 260 | 254.3 | 3 |
| | | | | | 25 |
| A | Dec 1/57 | 1 | 34 - 50 | 40.4 | 12 |
| | | 2 | 85 - 87 | 91.0 | 3 |
| | | 3 | 142 - 147 | 145.0 | 3 |
| | | 4 | 186 - 195 | 192.0 | 5 |
| | | 5 | 235 - 251 | 244.0 | 4 |
| | | 6 | 223 - 239 | 231.7 | 7 |
| | | | | | 34 |
| B | Mar 15/28 | 1 | 40 - 43 | 40.9 | 9 |
| | | 2 | 90 - 95 | 92.2 | 5 |
| | | 3 | 148 - 149 | 148.5 | 2 |
| | | 4 | 190 - 194 | 192.6 | 5 |
| | | 5 | 244 - 246 | 245.0 | 2 |
| | | | | | 23 |

completed until about February of the succeeding year. In P. marinus larvae, although metamorphosis begins at approximately the same time of year as it does in the other two species, it is accomplished considerably more quickly, the newly transformed adults appearing in mid-Winter. This is born out in Sample C, in which only five Size Classes can be segregated, the assumption being that during the preceding Winter the ammocoetes of Size Class 6 completed their metamorphosis and left the streams as adult lampreys.

Before any hard and fast conclusions could be drawn from these estimates of rates of growth in ammocoetes, ammocoetes had to be reared under control conditions in the laboratory in order to verify the field data. However, even with this tentative information as it stands, several possible conclusions might be suggested.

Firstly, although L. fluviatilis appears to re:main in the larval state for a year longer than does L. planeri, the respective average lengths of these two species during metamorphosis are not so different as one might be led to suppose. Thus the currently popular practise of attempting to determine the species of meta:morphosing ammocoetes by reference to their relative sizes is not accurate when these two species are concerned. Metamorphosing Sea Lamprey ammocoetes, on the other hand, are significantly larger than are ammocoetes of the other two species at a similar stage of development.

Secondly, it seems reasonable to conclude from the data obtained that ammocoetes of all three species do not undergo important increase in length during the winter months. This is probably associated with a decrease in feeding during cold weather.

LABORATORY EXPERIMENTS ON RATES OF GROWTH:

In April 1958, 10 ammocoetes of each size class of each species (except Size Class 1 animals, e.g. those spawned in the spring of 1958) were placed in acquaria provided with running water and several inches of soft mud. On the first day of each of thirteen ensuing months the length of every one of these specimens was recorded.

Ammocoetes were measured by placing them in test-tubes filled with water and sliding narrow rulers in beside them. This was an ideal method because the narrow-:ness of the tubes prevented the ammocoetes from bending into loops and the rulers gently pressed against the specimens reduced their wriggling movements to a minimum so that accurate readings ($\pm$1 millimetre) could be taken.

Length measurements of each size class of each species were averaged monthly. Decimal values were eliminated by raising the final integer by unity if the decimal was in excess of 0.5 and likewise ignoring the decimal altogether if it was less than 0.5. This was done because the readings were taken on a $\pm$1 millimetre level anyway, so that decimal values could certainly not be considered significant.

During the course of this test 3 Size Class 4 animals (1 L. fluviatilis and 3 L. planeri), as well as 2 Size Class 5 ammocoetes (L. planeri) died.

Originally it had been planned to measure rates of linear growth in ammocoetes of Size Class 1 in the same fashion. However, when the first specimens of this size level were removed from the tank in which they were spawned it was found that their small size and extreme activity rendered anything approaching accurate measure-:ment impossible. Therefore, instead of attempting live measurements, 3 of these ammocoetes were removed monthly from the spawning tank and were killed by contact with formalin before measurements were taken. In this way no single Size Class 1 ammocoete was ever measured twice, and also dependence was placed on rather small samples. Thus the data obtained for first year ammocoetes is only slightly more relevant than that derived from the field sampling methods of estimating growth.

Results of this experiment are shown in Tables XI - XIII and graphically in Figs. 10 - 15. The first note-:worthy fact indicated by these data are that the estimates of size classes in the field must have been fairly accurate, because after a year's growth under laboratory

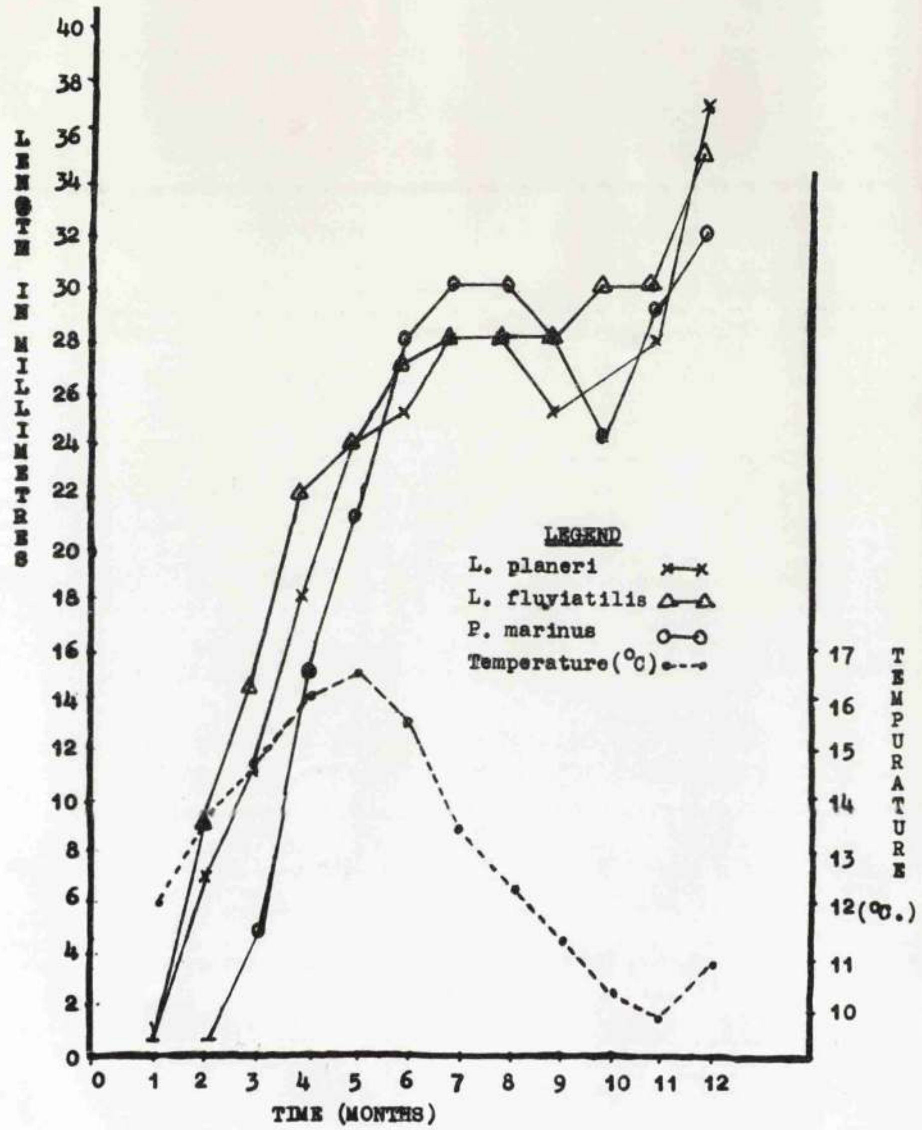


Fig. 10: Graph showing the rate of increase in body length of ammocoetes of L. planeri, L. fluviatilis and P. marinus during their first year of life, plotted against fluctuations in the temperature of the water in which they were kept.

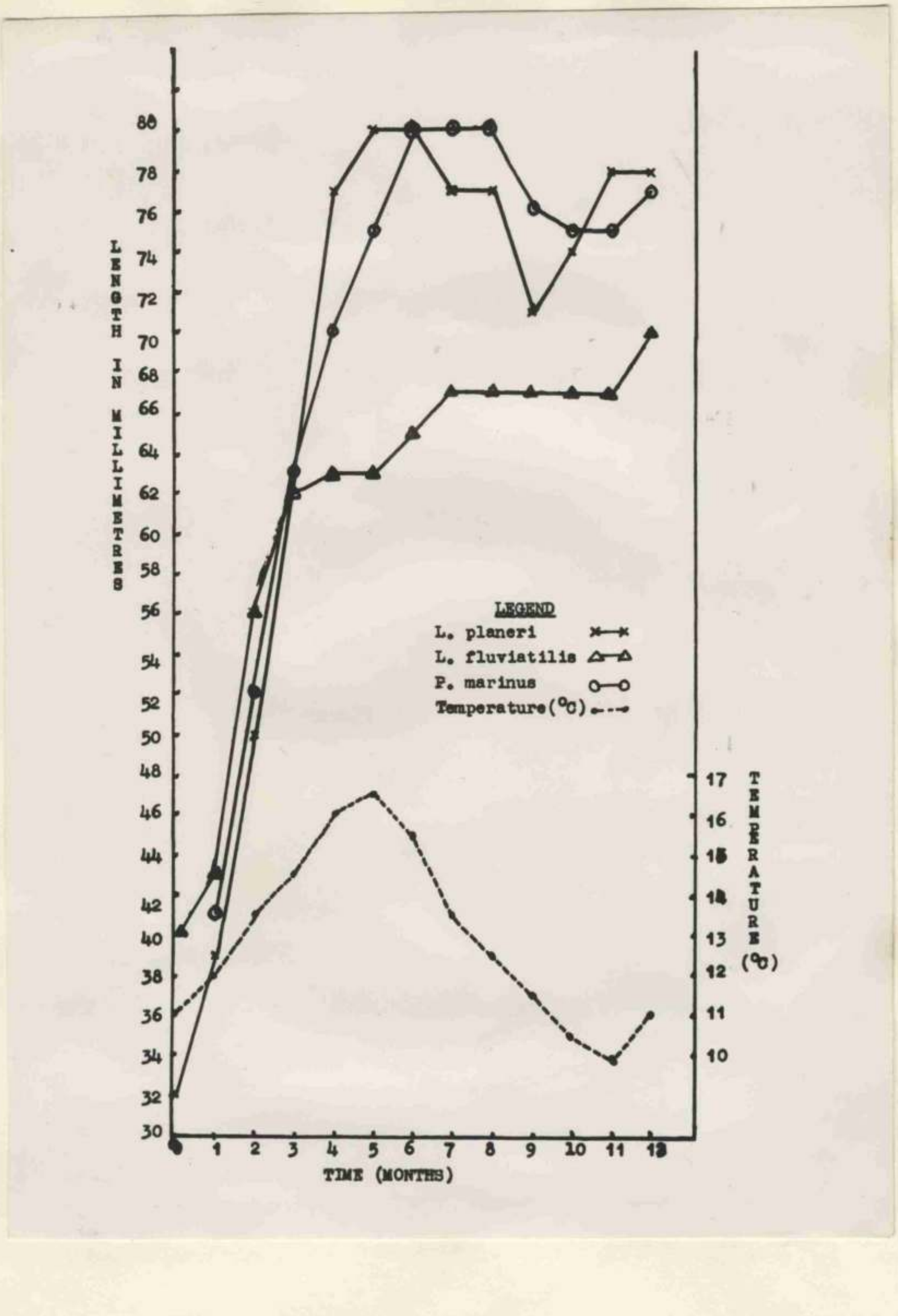


Fig. 11: Graph showing the rate of increase in body length of ammocoetes of L. planeri, L. fluviatilis and P. marinus during their second year of life, plotted against fluctuations in the temperature of the water in which they were kept.

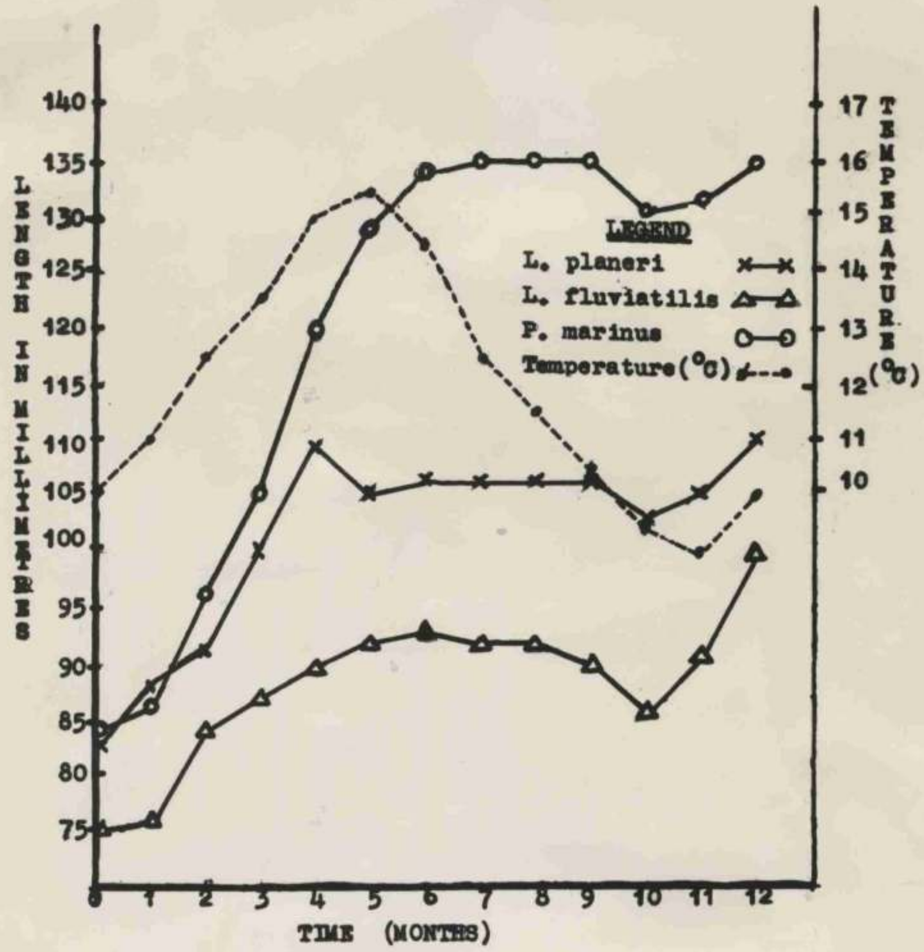


Fig. 12: Graph showing the rate of increase in body length of ammocoetes of L. planeri, L. fluviatilis and P. marinus during their third year of life, plotted against fluctuations in the temperature of the water in which they were kept.

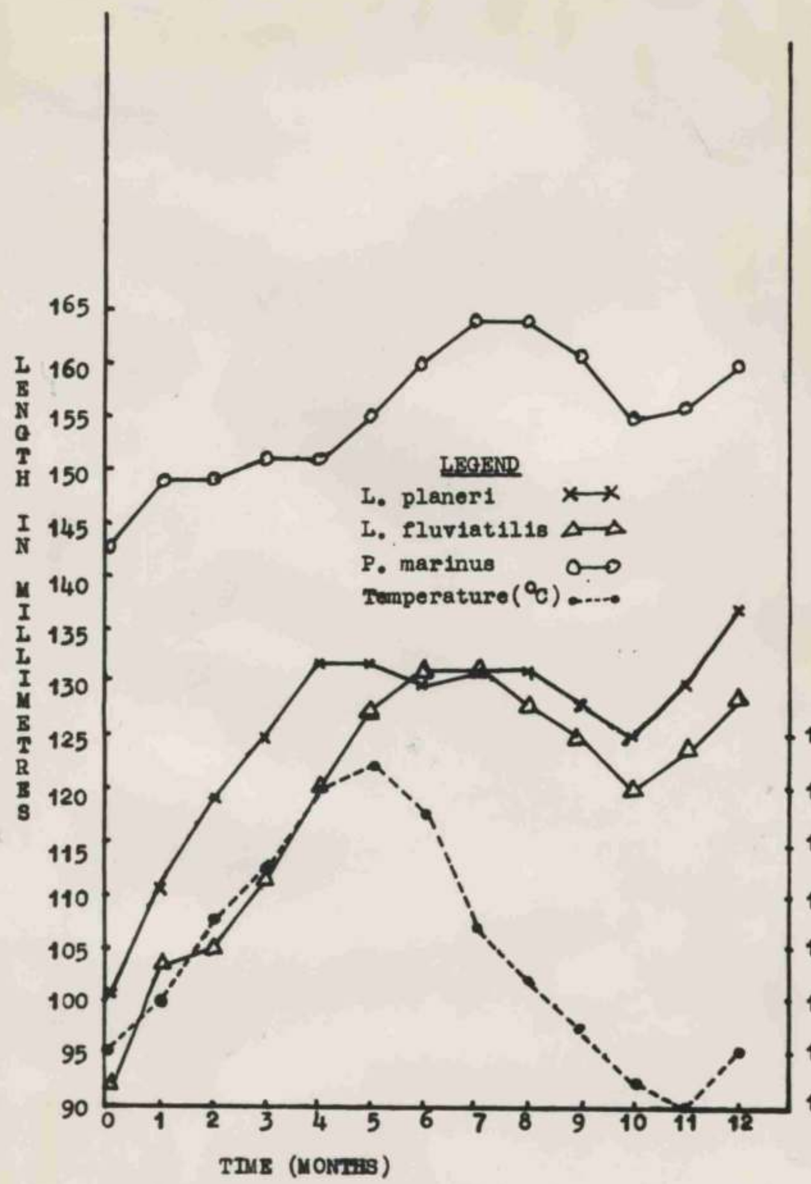


Fig. 13: Graph showing the rate of increase in body length of ammocoetes of L. planeri, L. fluviatilis and P. marinus during their fourth year of life, plotted against fluctuations in the temperature of the water in which they were kept.

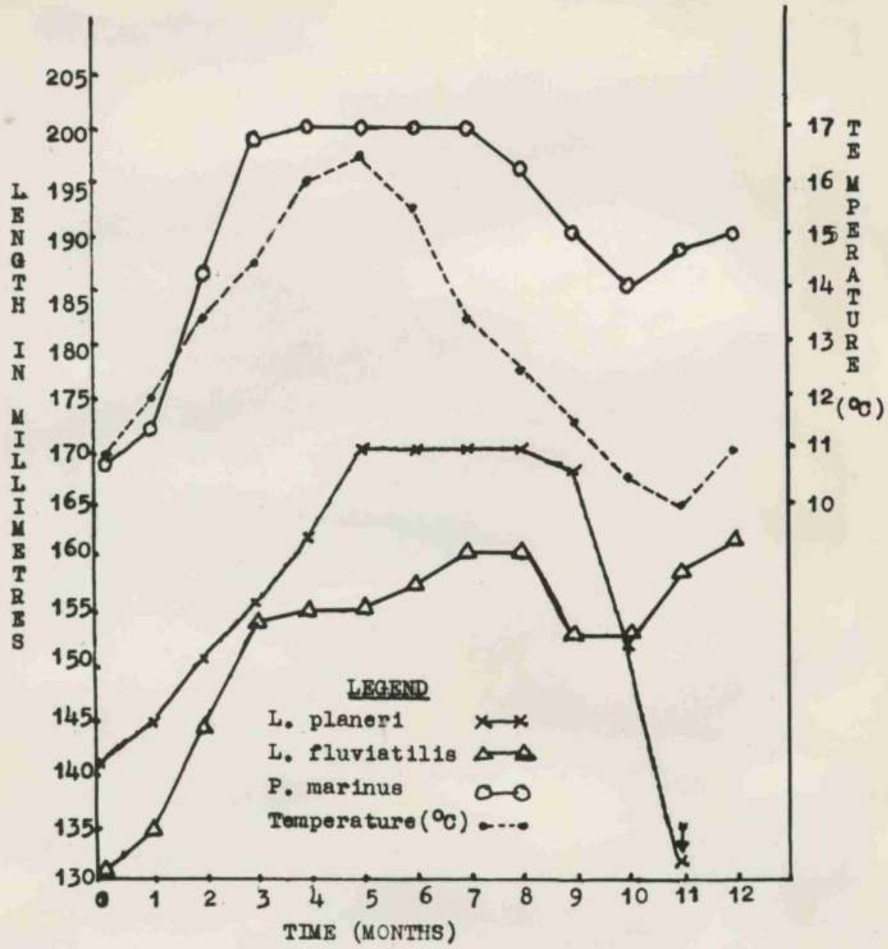


Fig. 14: Graph showing the rate of increase in body length of ammocoetes of L. planeri, L. fluviatilis and P. marinus during their fifth year of life, plotted against fluctuations in the temperature of the water in which they were kept. The arrow indicates the completion of metamorphosis in L. planeri.

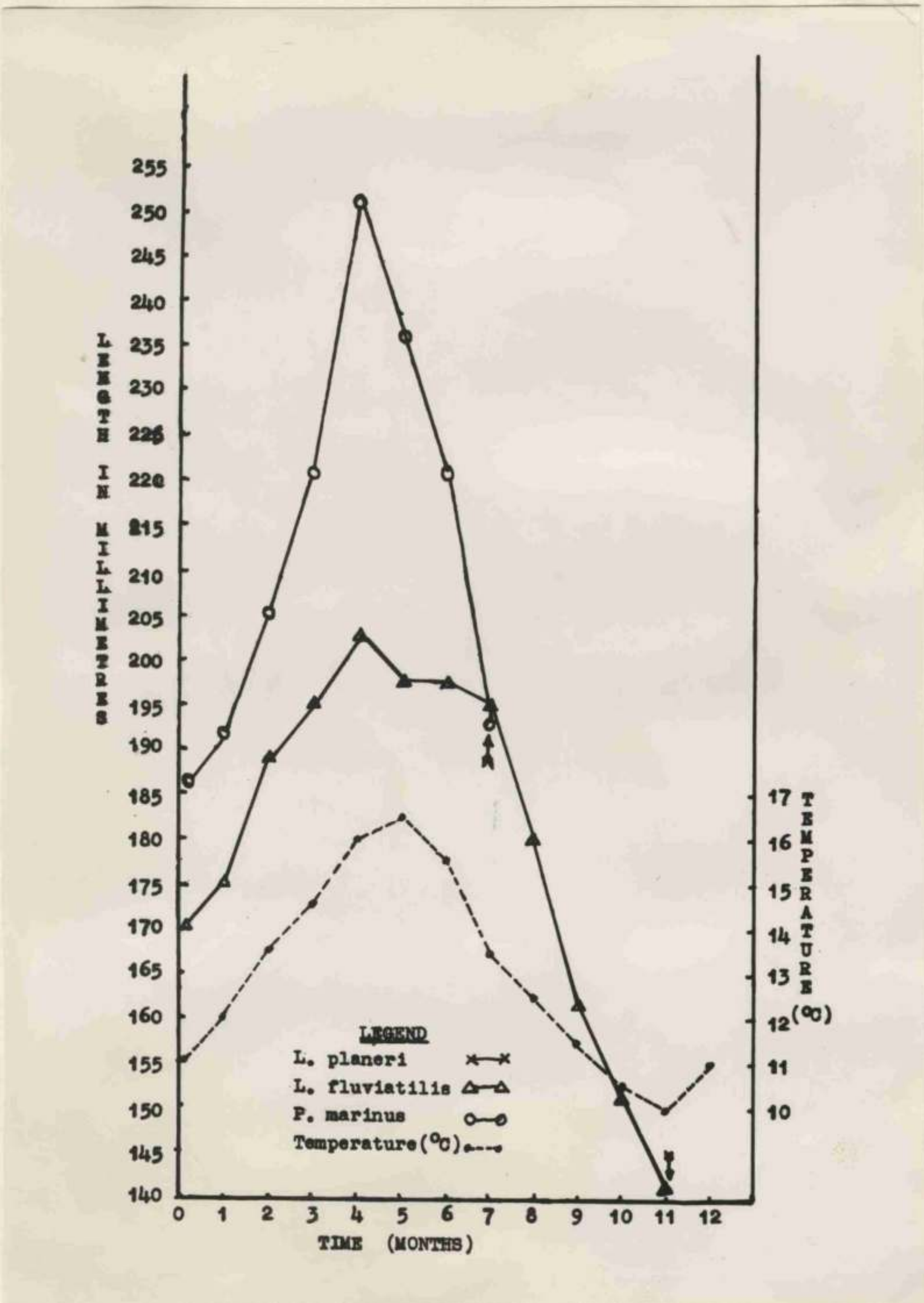


Fig. 15: Graph showing the rate of increase in body length of a mmocoetes of L. fluviatilis and P. marinus during their sixth year of life, plotted against fluctuations in the temperature of the water in which they were kept. The arrows indicate the completion of metamorphosis.

conditions the size classes do not overlap significantly. The biggest overlap (see Size Class 3, month 13: Table XII) was 9 millimetres, and the biggest deficit was 9 milli:metres (Table XII, Size Class 6, month 13). These differences can be ascribed to the fact that there is probably a slight variation in rate of growth in these animals from year to year due to temperature fluctuations etc. which would occur under natural conditions.

This means, then, that not only was the length of larval life of these three species more than likely correctly determined, but is also an indication that ammocoetes can be kept in captivity in this manner with:out grossly interfering with their normal rates of develop:ment.

In determining whether or not a correlation existed between temperature and rate of growth, a careful record of average daily water temperatures was kept. At the end of every month these figures were averaged so that temperatures shown in Tables XI-XIII and Figs. 10-15 for every month are in reality the average temperatures for the months preceding. That is, the ammocoetes were measured generally at the fifth of every month, while the average water temperature was recorded at the end of every month, the purpose of this being to see if ammocoete size at the beginning of one month bears any relationship to the temperatures which it had encountered throughout the previous month of growth.

A cursory glance at the 6 graphs (Figs. 10 - 15) suggests that such a correlation definitely exists. This is so to the surprising extent that, except in the cases of Size Classes 1 and 2 in L. fluviatilis, ammocoetes actually undergo a decrease in body length during the colder months when the water temperature is dropping.

As implied earlier, the data obtained under laboratory conditions upheld the inferences drawn from results achieved by sampling in the field. L. planeri ammocoetes metamorphose after approximately 55 months. This metamorphosis commences in late summer (about August) and finishes early in the succeeding Spring (about February). On the other hand, although P. marinus ammocoetes begin to metamorphose at approximately the same time of year as L. planeri larvae, the process in the former is accelerated so that they attain adulthood sometime in mid-Winter, after about 63 months of larval life. Ammo-:coetes of L. fluviatilis have a longer larval life than either Brook or Sea Lamprey larvae, for metamorphosis is not completed in this species until about the 67th. month. The process of metamorphosis takes as long in River Lamprey larvae as it does in those of the Brook Lamprey. Likewise it begins in August or thereabouts, terminating in adulthood sometime in February or March.

It will be remembered that in analyzing the ammocoetes taken in field samples, some confusion was encountered in assessing size classes (Pp. 54) due to the fact that certain specimens, while appearing to be more advanced morphologically than the others, were not the largest individuals. It was, of course, finally assumed that the largest ammocoetes were not necessarily the oldest, and that a shrinkage must occur during the last months of larval growth. The data derived from the laboratory experiment bears this out, as demonstrated graphically in Figs. 10-15. It is assumed that the shrinkage is associated with starvation during metamor-:phosis.

TABLE XI - RATES OF GROWTH OF L. planeri AMMOCOETES IN LABORATORY.

| No. of Months Growth | Date | $SC_1$ | $SC_2$ | $SC_3$ | $SC_4$ | $SC_5$ | Monthly Average Water Temp. (°C) |
|---|---|---|---|---|---|---|---|
| 0 | Apr. 5 | 0 | 32 | 83 | 102 | 141 | 11.0 |
| 1 | May 5 | ns | 39 | 87 | 111 | 145 | 12.0 |
| 2 | June 5 | 7 | 50 | 92 | 118 | 151 | 13.5 |
| 3 | July 5 | 11 | 63 | 100 | 125 | 156 | 14.5 |
| 4 | Aug. 5 | 18 | 77 | 109 | 132 | $163^{x}$ | 16.0 |
| 5 | Sept. 5 | 24 | 81 | 105 | 132 | $170^{x}$ | 16.5 |
| 6 | Oct. 5 | 25 | 80 | 106 | 130 | $170^{x}$ | 15.5 |
| 7 | Nov. 5 | 28 | 77 | 106 | 131 | $170^{x}$ | 13.5 |
| 8 | Dec. 5 | 28 | 77 | 106 | 131 | $170^{x}$ | 12.5 |
| 9 | Jan. 22 | 25 | 71 | 106 | 128 | $167^{x}$ | 11.5 |
| 10 | Feb. 5 | 24 | 74 | 104 | 125 | $153^{x}$ | 10.5 |
| 11 | Mar. 5 | 28 | 78 | 105 | 130 | $132^{xx}$ | 10.0 |
| 12 | Apr. 3 | 37 | 78 | 109 | 137 | | 11.0 |

"ns" means that the specimens are just developing from egg.

"x" means that metamorphosis is taking place.

"xx" means that metamorphosis is complete.

TABLE XII - RATES OF GROWTH IN AMMOCOETES OF L. fluviatilis IN LABORATORY.

| No. of Months Growth | Date | $SC_1$ | $SC_2$ | $SC_3$ | $SC_4$ | $SC_5$ | $SC_6$ | Monthly Average Water Temp. ($^oC$) |
|---|---|---|---|---|---|---|---|---|
| 0 | Apr. 5 | 0 | 40 | 75 | 91 | 131 | 170 | 11.0 |
| 1 | May 5 | ns | 43 | 76 | 103 | 135 | 175 | 12.0 |
| 2 | June 5 | 9 | 56 | 84 | 105 | 144 | 189 | 13.5 |
| 3 | July 5 | 14 | 62 | 87 | 111 | 154 | 195 | 14.5 |
| 4 | Aug. 5 | 22 | 63 | 89 | 120 | 155 | 203 | 16.0 |
| 5 | Sept. 5 | 24 | 63 | 92 | 127 | 155 | $198^x$ | 16.5 |
| 6 | Oct. 5 | 27 | 65 | 93 | 131 | 157 | $198^x$ | 15.5 |
| 7 | Nov. 5 | 28 | 67 | 92 | 131 | 160 | $195^x$ | 13.5 |
| 8 | Dec. 5 | 28 | 67 | 92 | 128 | 160 | $180^x$ | 12.5 |
| 9 | Jan. 22 | 28 | 67 | 90 | 125 | 153 | $162^x$ | 11.5 |
| 10 | Feb. 5 | 29 | 67 | 86 | 120 | 153 | $151^x$ | 10.5 |
| 11 | Mar. 5 | 29 | 67 | 91 | 124 | 158 | $141^{xx}$ | 10.0 |
| 12 | Apr. 3 | 35 | 70 | 100 | 128 | 161 | | 11.0 |

"ns" means that ammocoete is just developing from egg.

"x" means that metamorphosis is taking place.

"xx" means that metamorphosis is complete.

TABLE XIII - RATES OF GROWTH OF P. marinus AMMOCOETES IN LABORATORY.

| No. of Months Growth | Date | | $SC_1$ | $SC_2$ | $SC_3$ | $SC_4$ | $SC_5$ | $SC_6$ | Monthly Average Water Temp. ($^oC$) |
|---|---|---|---|---|---|---|---|---|---|
| 0 | Apr. | 5 | 0 | 40 | 84 | 143 | 169 | 186 | 11.0 |
| 1 | May | 5 | 0 | 41 | 86 | 148 | 172 | 192 | 12.0 |
| 2 | June | 5 | ns | 52 | 97 | 148 | 186 | 205 | 13.5 |
| 3 | July | 5 | 5 | 63 | 105 | 151 | 199 | 221 | 14.5 |
| 4 | Aug. | 5 | 15 | 70 | 120 | 151 | 200 | $251^{x}$ | 16.0 |
| 5 | Sept. | 5 | 21 | 75 | 129 | 155 | 200 | $236^{x}$ | 16.5 |
| 6 | Oct. | 5 | 28 | 80 | 134 | 160 | 200 | $221^{x}$ | 15.5 |
| 7 | Nov. | 5 | 30 | 80 | 135 | 164 | 200 | $194^{xx}$ | 13.5 |
| 8 | Dec. | 5 | 30 | 80 | 135 | 164 | 196 | | 12.5 |
| 9 | Jan. | 22 | 28 | 76 | 135 | 161 | 190 | | 11.5 |
| 10 | Feb. | 5 | 24 | 75 | 131 | 155 | 185 | | 10.5 |
| 11 | Mar. | 5 | 29 | 75 | 132 | 156 | 188 | | 10.0 |
| 12 | Apr. | 5 | 32 | 77 | 135 | 160 | 190 | | 11.0 |

"ns" means that the specimen is just developing.

"x" means that metamorphosis is taking place.

"xx" means that metamorphosis is complete.

VARIATIONS IN RATE OF LINEAR GROWTH OF *L. planeri* AMMOCOETES:

Although the data obtained by Hardisty (1951) on *L. planeri* agrees closely with the author's own data on the same species in Scotland, there exists in the literature considerable disagreement about the length of larval life in Brook Lampreys. Hardisty (1951) demonstrated that slight variations occur from one stream to another.

After establishing that water temperature is intimately correlated with rate of growth, the present author wished to determine whether significant variations in rates of ammocoete growth could be ascribed to important climatic differences, especially temperature, in various regions.

The two most dependable contributions on rates of growth in *L. planeri* ammocoetes previous to the present paper, are those by Schultz (1930), in which ammocoetes from Washington State, U.S.A., are discussed, and Hardisty (1951), in which ammocoetes from Somerset in England are discussed. In such important climatic considerations as annual variation in temperature, mean summer and winter temperatures, and amount of rainfall, there is a great similarity between England and Washington.*

By way of contrast, the present author collected a sample of L. planeri ammocoetes from the North Knife River, which drains into Hudson's Bay in the Canadian Arctic.

Adult L. planeri were not observed to spawn in that river until mid-June. Schultz (1930), working in Washington, Hardisty (1944), working in England, and MacDonald (1959), working in Scotland, all agreed that L. planeri spawned in each of the areas concerned, in the latter half of April or early in May. These same three investigators also agreed on the optimum water temperature for spawning (i.e. 10 - 12°C).

When spawning of Brook Lamprey was first observed by the author in the North Knife River on June 17, 1959, the water temperature was 11°C.

*This information was supplied through the courtesy of the U.S. Meteorological Bureau, Washington, D.C., by private correspondence.

This, then, would indicate rather strongly, that water temperature, rather than the time of year, is the dominant factor in determining when spawning of L. planeri shall occur.

A single sample of L. planeri ammocoetes was taken from the North Knife River on August 10, 1959, at which time the water temperature was 12°C. The ammocoetes were measured in the usual manner to the nearest millimetre, and the data derived was as follows:

9, 12, 14(8), 15, 17(11), 18, 21, 44, 45(3), 46(2), 47(15), 50, 68(6), 71, 72, 75(4), 76, 78, 80(3), 90, 91, 93(7), 95, 108, 111(9), 115, 119, 120(5), 150,* 151,* 153,* 165, 167, 168, 172.

Three of the specimens, namely those designated by asterisks, had almost completed metamorphosis. Analy-:sis of the data (see Table XIV) shows seven discreet size classes.

It will be noticed in Table XIV that the ammo-:coetes which were in advanced stages of metamorphosis were treated as a discreet group and were placed last on the list as they were obviously the oldest specimens.

It has been shown earlier in this paper that once the water temperature drops much below 8 or 9°C, very little, if any growth takes place in ammocoetes. The North Knife River does not thaw out until May. During June and July the water temperature rises rapidly due to the intense heat of the brief arctic summer. Towards the end of August the air temperature tends to drop precipitously. During the summer of 1959, for example, it snowed three times in the last week of August. By mid-September ice has generally formed in all but the swiftest parts of the North Knife River, at which time it can safely be assumed that ammocoete growth has come to an end for the season.

It can be seen, therefore, that while ammocoetes of L. planeri in Britain have a growing season of seven or eight months, those in the North Knife River have only about three or four months at the most. During the arctic summer the water temperature in the North Knife River ranges from about 8°C to about 19°C. The rate of growth of ammocoetes in that river throughout any given week of the growing season is hence probably not very different from that in British streams. Therefore, it can be expected that, since L. planeri ammocoetes in the North

TABLE XIV - SHOWING THE SIZE CLASSES DERIVED FROM ONE SAMPLE OF AMMOCOETES OF L. *planeri* FROM THE NORTH KNIFE RIVER ON AUGUST 10, 1959. (Measurements in Millimetres)

| Size Class | Range in Length | Average Length | Number of Specimens |
|---|---|---|---|
| 1 | 9 - 21 | 15.8 | 24 |
| 2 | 44 - 50 | 43.5 | 22 |
| 3 | 68 - 80 | 73.2 | 17 |
| 4 | 90 - 95 | 92.7 | 10 |
| 5 | 108 - 120 | 114.2 | 17 |
| 6 | 165 - 172 | 168.0 | 4 |
| 7 | 150 - 153 | 151.3 | 3 |

Knife River metamorphose at about the same body length as their British counterparts, the former require more growing seasons in which to attain metamorphosis than do the latter.

For this reason, the seven size classes segregated in Table XIV can probably be regarded as year classes, indicating that *L. planeri* from the North Knife River in the Arctic, have a larval life of about two to three years in excess of *L. planeri* from the temperate zone.

AGE, WEIGHT AND LENGTH:

In order to establish what relationships, if any, existed between the approximate age, weight and body length of ammocoetes, the specimens used in the laboratory rates of growth analysis were killed and weighed. Also ammocoetes of approximately four months age, which had been preserved for almost a year in 70% alcohol, were dried off and weighed. All weighing was done on analytic balances giving accurate readings to a hundredth of a milligram.

The results of this test are shown for each species in Tables XV - XVII.

The most outstanding fact which comes to light in this analysis is that in all three species concerned, weight increases at a far greater rate than does length as ammocoetes gain in age. Of course, this follows in:tuitively, as the girth of an ammocoete increases as it grows.

Also, the supposition made earlier in this paper (Pp. 72) that the decrease in length of metamor:phosing ammocoetes is due to a cessation of feeding is amply supported by the fact, clearly shown in this data, that a decrease in body weight takes place during meta-:morphosis in all three species.

Ranges in weight within each sample of ten ammocoetes were insignificantly small, indicating that ammocoetes of similar age, and living under almost identi-:cal conditions, feed at approximately the same rate.

Speaking from the point of view of comparisons between species, the results of this test were most in-:teresting. Reference to Tables XV - XVII show that small-:er ammocoetes (those within the first 16 months of life) of all three species follow similar growth-feeding patterns in that the body weight increases at a greater rate then than it does at any other stage of larval development. In larvae of P. marinus this tendency appears to be some-:what less marked than it is in the other two species.

In the following year of larval life, ammocoetes of P. marinus and L. planeri increase in weight at approxi-:mately the same rate, while ammocoetes of L. fluviatilis gain weight at a slightly greater rate. From the beginning of the subsequent year on through the next two, however, there are wide differences between the species in the relationship between age and weight.

While *L. fluviatilis* larvae continue to increase in weight at approximately the same rate, *P. marinus* ammocoetes do so at a greater rate than they had in the previous year, while ammocoetes of *L. planeri* manifest a very sudden increase in body weight.

In the next year, the larvae of *L. fluviatilis* and *P. marinus* continue to increase in weight at about the same rate as they do during the previous year, while ammocoetes of *L. planeri* show a sudden decrease in rate of gain of weight. This decrease is so abrupt that *L. planeri* ammocoetes of about four years of age are lighter than *P. marinus* larvae of the same age, whereas, until this decrease comes about, the former are considerably heavier than similar age groups of the latter.

In the final two years of life, *P. marinus* ammocoetes show a decline in rate of weight gain, while larvae of *L. fluviatilis* show no change in this regard.

The pattern of weight loss during the last few months of metamorphosis appears to be consistent with the attendant decrease in length in all three species. During this period, *L. fluviatilis* undergo a decrease in length of about 50 millimetres, accompanied by a weight loss of about 2,211 milligrams. L. planeri ammocoetes, on the other hand, lose only about 22 millimetres, this being attended by a decrease in body weight of 1,322 milligrams. Ammocoetes of P. marinus, while manifesting the least decrease in length during metamorphosis (an average of 16 millimetres), likewise lose the least weight (about 368 milligrams).

TABLE XV - APPROXIMATE RELATIONSHIP BETWEEN AGE AND LENGTH AND WEIGHT IN AMMOCOETES OF *L. planeri*.

| Approx. Age (Months) | Average Length (Millimetres) | Average Weight (Milligrams) | Range in Weight (Milligrams) | Number of Specimens |
|---|---|---|---|---|
| 4 | 15 | 13.11 | 12.90 - 14.00 | 10 |
| 16 | 77 | 207.15 | 200.00 - 209.75 | 10 |
| 28 | 109 | 2003.89 | 1992.50 - 2017.50 | 10 |
| 40 | 132 | 2245.57 | 2233.00 - 2573.19 | 10 |
| 52 | 163 | 4619.66 | 4575.50 - 4752.90 | 10 |
| 59 | 148 | 3296.38 | 3061.00 - 3371.28 | 10 |
| | | | | 60 |

TABLE XVI - APPROXIMATE RELATIONSHIP BETWEEN AGE AND LENGTH AND WEIGHT IN AMMOCOETES OF *L. fluviatilis*.

| Approx. Age (Months) | Average Length (Millimetres) | Average Weight (Milligrams) | Range in Weight (Milligrams) | Number of Specimens |
|---|---|---|---|---|
| 4 | 20 | 18.50 | 18.00 - 19.00 | 10 |
| 16 | 69 | 297.21 | 215.95 - 342.31 | 10 |
| 28 | 92 | 1931.20 | 1850.00 - 2055.43 | 10 |
| 40 | 118 | 2913.60 | 2900.00 - 3014.90 | 10 |
| 52 | 163 | 4925.29 | 4813.26 - 5099.40 | 10 |
| 64 | 211 | 6991.15 | 6900.75 - 7103.87 | 10 |
| 71 | 151 | 4780.32 | 4518.90 - 4821.33 | 10 |
| | | | | 70 |

TABLE XVII - APPROXIMATE RELATIONSHIP BETWEEN AGE AND LENGTH AND WEIGHT IN AMMOCOETES OF *P. marinus*.

| Approx. Age (Months) | Average Length (Millimetres) | Average Weight (Milligrams) | Range in Weight (Milligrams) | Number of Specimens |
|---|---|---|---|---|
| 4 | 15 | 22.50 | 20.35 - 23.75 | 10 |
| 16 | 79 | 1191.20 | 1021.80 - 1306.48 | 10 |
| 28 | 114 | 2595.00 | 2539.00 - 2611.98 | 10 |
| 40 | 165 | 3895.32 | 3858.60 - 4022.80 | 10 |
| 52 | 212 | 6207.63 | 6041.80 - 6219.35 | 10 |
| 64 | 240 | 7482.50 | 7472.00 - 7501.50 | 10 |
| 67 | 234 | 7114.09 | 7690.40 - 8129.25 | 10 |
| | | | | 70 |

## EFFECTS OF EXTREME TEMPERATURES ON RATES OF FEEDING AND GROWTH IN AMMOCOETES:

Several attempts were made to maintain living ammocoetes of various sizes and species at the maximum water temperature encountered in the laboratory (19°C) over a period of one year. These tests inevitably resulted in the death of the larvae within a few months of the time (about September) when the water temperature in the laboratory normally begins to drop from its maximum summer level.

Likewise, attempts to keep ammocoetes at the minimum water temperature encountered in the laboratory (10°C) for much more than several weeks resulted in the death of all specimens tested.

Generally speaking it was noticed that ammocoetes killed by exposure to prolonged maximum temperatures appeared to be considerably larger and heavier than normal ammocoetes of similar age, while those killed by prolonged exposure to a minimum seemed to be lighter in weight than one would expect larvae of that same age and species to be.

It was also noticed that all sizes of ammocoetes tested, of all three species, were more sensitive to minimum than to maximum water temperatures. On the basis of these findings it was assumed that an increase in water temperature brings about an increase in rate of feeding of ammocoetes, while a decrease in water temperature causes the reverse effect.

In order to test this hypothesis more fully, the following experiment was conducted. At a time of year when neither extreme of water temperature was being nor:mally encountered (October) several days were spent in an intensive ammocoete collecting program in order to obtain six specimens of each size class and of each species. Three acquaria were then set up in the usual fashion, two ammocoetes of each size class and species (a total of 34 specimens) being placed in each. The tanks were designated by the letters A, B, and C.

By artificially raising the water temperature in tank A through 1°C a day, until a temperature of 19°C was reached, the ammocoetes were acclimatized to the maxi:mum water temperature. Similarly, by lowering the water temperature in tank B through 1°C a day until a tempera:ture of 10°C was recorded, ammocoetes in that tank were acclimatized to a minimum water temperature. The ammocoetes in tank C were used as a control group.

Two days were then spent in measuring the living ammocoetes in all three tanks (method described on Pp. 68), and in weighing them. The latter was accom:plished as follows. A 250 cc. cone flask containing exactly 50 cc. of water (as measured by Burette) was first weighed on the analytic balance, a weight of 180.54 grams being recorded.

Then ammocoetes were removed from the tanks one at a time, rolled quickly across a strip of gauze (to remove excess moisture) and transferred into the cone flask containing the 50 cc. of water. Each ammocoete was thus weighed on the same balance separately, the known weight of the flask and water being subtracted in each instance from the total weight recorded.

This accomplished, the ammocoetes were restored to their respective tanks, and were left undisturbed for the next 60 days. At the end of this time they were all again weighed and measured.

Data derived from these experiments are shown in Tables XVIII - XXI. Even a cursory glance at the figures shows that there was definitely an increase in both weight and length in those ammocoetes kept at 19°C.

water temperature. In L. planeri and P. marinus ammocoetes, an ascending order of weight increases, starting from the smallest and passing up to the largest size class, was noted. While the larvae of L. fluviatilis did not follow this pattern exactly, the same general tendency was obvious. See Table XVIII.

Size Class I ammocoetes of all three species were approximately the same length at the start of the experiment, and increased in both weight and length by close to the same amount during the ensuing 60 days in tank A. A comparison of rates of feeding (as indicated by increase in body weight) as related to increase in body length, between the three species of ammocoetes is illustrated in Tables XIX - XXI.

A study of the data from tank B (10°C) indicates that there is some shrinkage and loss of weight in most of the size groups tested. This implies that a cessation of feeding is associated with low water temperatures. The fact that 12 of the 34 ammocoetes used in this experiment died, shows that lamprey larvae are rather more sensitive to minimum than to maximum water temperatures. Also those that did die were (except for two) within

18 months of metamorphosis. All the final year animals in tank B died before the completion of the 60 day test, and when found, their corpses had undergone such decom:position that it would have been futile to carry out a weight-length analysis of them.

The larvae in tank C (Control Group) did not deviate significantly from the normal growth patterns as determined previously in this paper (Pp. 68 - 75).

TABLE XVIII - AMOUNT OF WEIGHT INCREASE AFTER 60 DAYS IN TANK A (Milligrams).

| Size Class | L. planeri | L. fluviatilis | P. marinus |
|---|---|---|---|
| 1 | 6 | 8 | 8 |
| 2 | 8 | 22 | 64 |
| 3 | 16 | 16 | 161 |
| 4 | 109 | 18 | 192 |
| 5 | 313 | 115 | 294 |
| 6 | | 515 | 365 |

TABLE XIX - SHOWING RELATIONSHIP BETWEEN WATER TEMPERATURE AND LENGTH AND WEIGHT IN AMMOCOETES OF *L. planeri*.

| Size Class | Tank A (19°C) | | | | Tank B (10°C) | | | | Tank C (11 - 12°C) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $L_1$ | $L_2$ | $W_1$ | $W_2$ | $L_1$ | $L_2$ | $W_1$ | $W_2$ | $L_1$ | $L_2$ | $W_1$ | $W_2$ |
| 1 | 27 | 31 | 29.20 | 35.50 | 26 | 24 | 28.00 | 25.55 | 27 | 27 | 31.00 | 31.90 |
| 2 | 80 | 86 | 232.75 | 241.00 | 79 | 75 | 229.15 | 206.40 | 80 | 81 | 240.15 | 241.50 |
| 3 | 102 | 107 | 1998.50 | 2015.20 | 104 | 102 | 2012.20 | 1983.50 | 104 | 105 | 1830.00 | 1840.75 |
| 4 | 123 | 130 | 2290.25 | 2399.10 | 123 | 119 | 2305.70 | 2007.50 | 122 | 122 | 2275.10 | 2276.09 |
| 5 | 166 | 172 | 4500.50 | 4813.25 | 167 | died | 4670.85 | | 167 | 166 | 4722.95 | 4599.50 |

LEGEND

$L_1$ means Length of Ammocoete at Beginning of Experiment (millimetres).

$L_2$ means Length of Ammocoete at End of 60 days (millimetres).

$W_1$ means Weight of Ammocoete at Beginning of Experiment (milligrams).

$W_2$ means Weight of Ammocoete at End of 60 days (milligrams).

TABLE XX - SHOWING RELATIONSHIP BETWEEN WATER TEMPERATURE AND LENGTH AND WEIGHT IN AMMOCOETES OF *L. fluviatilis*.

| Size Class | Tank A (19°C) | | | | Tank B (10°C) | | | | Tank C (11 - 12°C) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $L_1$ | $L_2$ | $W_1$ | $W_2$ | $L_1$ | $L_2$ | $W_1$ | $W_2$ | $L_1$ | $L_2$ | $W_1$ | $W_2$ |
| 1 | 27 | 34 | 49.50 | 57.50 | 27 | 27 | 48.15 | 49.00 | 27 | 29 | 49.20 | 50.05 |
| 2 | 69 | 77 | 302.25 | 323.90 | 72 | 70 | 311.35 | 303.10 | 72 | 74 | 319.45 | 335.80 |
| 3 | 90 | 99 | 1919.35 | 1935.20 | 87 | 86 | 1922.80 | 1901.75 | 91 | 93 | 1976.20 | 1990.52 |
| 4 | 128 | 138 | 3075.50 | 3093.55 | 128 | 128 | 3079.25 | 3071.30 | 128 | 131 | 3066.30 | 3085.77 |
| 5 | 160 | 166 | 4471.40 | 4586.15 | 160 | died | 4552.50 | | 160 | 161 | 4560.08 | 4569.85 |
| 6 | 195 | 199 | 6497.15 | 7011.95 | 197 | died | 6603.70 | | 195 | 197 | 6491.72 | 6505.10 |

LEGEND

$L_1$ means Length of Ammocoete at Beginning of Experiment (millimetres).

$L_2$ means Length of Ammocoete at End of 60 days (millimetres).

$W_1$ means Weight of Ammocoete at Beginning of Experiment (milligrams).

$W_2$ means Weight of Ammocoete at End of 60 days (milligrams).

TABLE XXI - SHOWING RELATIONSHIP BETWEEN WATER TEMPERATURE AND LENGTH AND WEIGHT IN AMMOCOETES OF *P. marinus*.

| Size Class | Tank A ($19^\circ C$) | | | | Tank B ($10^\circ C$) | | | | Tank C ($11 - 12^\circ C$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $L_1$ | $L_2$ | $W_1$ | $W_2$ | $L_1$ | $L_2$ | $W_1$ | $W_2$ | $L_1$ | $L_2$ | $W_1$ | $W_2$ |
| 1 | 25 | 31 | 35.00 | 42.60 | 25 | 24 | 34.90 | 30.27 | 25 | 26 | 32.50 | 33.80 |
| 2 | 87 | 99 | 1328.15 | 1391.80 | 84 | 80 | 995.33 | 978.75 | 84 | 85 | 1277.10 | 1320.50 |
| 3 | 112 | 121 | 2705.20 | 2866.25 | 115 | died | 2808.50 | | 111 | 111 | 2598.25 | 2617.12 |
| 4 | 155 | 165 | 3726.20 | 3918.40 | 155 | 148 | 3711.25 | 3693.70 | 156 | 157 | 3710.45 | 3713.05 |
| 5 | 202 | 215 | 6191.65 | 6485.00 | 199 | died | 6207.30 | | 205 | 205 | 6218.50 | 6215.70 |
| 6 | 258 | 265 | 7185.90 | 7550.65 | 258 | died | 7035.00 | | 260 | 260 | 7141.72 | 7132.90 |

LEGEND

$L_1$ means Length of Ammocoete at Beginning of Experiment (millimetres).

$L_2$ means Length of Ammocoete at End of 60 days (millimetres).

$W_1$ means Weight of Ammocoete at Beginning of Experiment (milligrams).

$W_2$ means Weight of Ammocoete at End of 60 days (milligrams).

## SUMMARY AND DISCUSSION OF GROWTH IN AMMOCOETES:

The data on growth embodied in Pp. 68 to 75 gives a quite clear picture of the rate at which ammo:coetes of the three species studied increase in length. If fluctuations in the body weight of these animals can be considered as an index of their rates of feeding, a considerable body of information about ammocoete feeding cycles can also be gleaned from the same pages.

Analysis of age groups, based on the length of the body (except in the case of metamorphosing ammocoetes, which were automatically considered as being in the final year of life, regardless of their size) yielded sufficient data to provide a framework for further, more accurate, analysis under laboratory conditions. The latter produced data which, not only strengthened tentative conclusions drawn from samples taken at random from streams, but indicated that:

(a) Ammocoetes in the last few months of larval life undergo a very marked decrease in length. This shrinkage appears to occur during declining water temperatures, not only in ammocoetes of P. marinus (which metamorphose during the early winter), but also in larvae of L. planeri and L. fluviatilis (which metamorphose in the early spring.

(b) Otherwise, water temperature and rate of linear growth in ammocoetes appear to be correlated.

To throw more light on both of these points, further tests were necessary. All ammocoetes used in the rates of linear growth experiment in the laboratory were weighed, as were some very young larvae, the latter being at about the fourth month of development. This was a prerequisite to the establishment of a certain criterion of normal weight-length relationships by which results of tests done at abnormal water temperatures could be judged.

It was realized that, although ammocoetes were shown by both field and laboratory analyses to grow only during the warmer months of the year and to undergo no increase in length, or even to shrink, during the cold months, the resulting correlation might not be merely a simple two way relationship between water temperature and rates of feeding. The possibility of inherent physiological mechanisms producing regular cycles of feeding and starving could not be overlooked.

To investigate this problem, a test with constant minimum and maximum water temperatures was carried out (Pp. 73) at a time of year when intermediate water temperatures were normally being encountered. Although this test suffered from the obvious defect of there being an insufficient number of ammocoetes available (only two in each sample), the results were rather definite in favour of the view that rate of feeding (hence rate of gain in weight and increase in length) is correlated, at least mainly, with the water temperature. If there is an internal physiological rhythm of feeding operating irregardless of water temperature, it is subordinate to maximum and minimum values of the latter.

SUGGESTIONS FOR FURTHER RESEARCH:

As is the way with the vast majority of research projects, the author feels that, while most of the answers sought for in this series of experiments have been answered satisfactorily within the present paper, the solutions themselves have brought to light an even greater host of unanswered questions.

The fact that identification of ammocoetes of Brook Lamprey (L. planeri) revealed that they were similar in every respect with larvae of the American Brook Lamprey (Entosphenus lammottenni), indicates the need for a complete re-evaluation of lamprey taxonomy. It has long been the author's suspicion that an understanding of the evolution and systematics of lampreys is badly hindered by bad taxonomy. This is particularly the case in America, where at least 20 generic names for various small lampreys, resembling the British Brook Lamprey, are extant. If most of these 'types' of lampreys were found to be identical with L. planeri, a great deal of useless nomenclature could be cleared away.

There exists the belief that L. planeri might be sexually precocious L. fluviatilis, and hence not really a species in its own right at all. Although the section on ammocoete identification in this paper gives fairly strong evidence for the view that the two really are separate species, as does the fact that their larval life spans differ in length by one year, the possibilities of hybridization between species of lampreys cannot be ignored. While working with the Fisheries Research Board of Canada, the author fertilized eggs of Sea Lamprey with spermatozoa from Brook Lampreys. Many of these eggs underwent further development, and some of the larvae were 35 days old when a poison was accidentally released into the tanks, bringing about the death of the young hybrids. In this country, E. W. Baxter (personal cor:respondence 1958) crossed _L. planeri_ with _L. fluviatilis_ both ways and obtained larvae. His experiment was also suddenly terminated by an unfortunate accident. Both of these incidents point up the pressing need for a well-defined research project on this topic.

An investigation of the mechanisms envolved in ammocoete metamorphosis would not be amiss. The present research indicates that each of the three species of lamprey studied metamorphose differently. _L. planeri_ metamorphoses in the Spring after close to 5 years of larval development. _L. fluviatilis_ does so after about 6 years of larval life. _P. marinus_ differs from both in that it metamorphoses in early Winter. The picture is further complicated by the fact that all three species begin to metamorphose at the same time of year (August-September), so that the period of metamorphosis is several months shorter in _P. marinus_ ammocoetes than it is in the other two species.

Also, research on this aspect of lamprey biology would be well worthwhile if an understanding of what follows metamorphosis is to be had. In L. planeri metamorphosis introduces the adult to a life of starvation, in which gonadal development takes place as the gut de-:generates. On the other hand, metamorphosis in L. fluviatilis and P. marinus initiates a predatory blood feeding life, in which the lamprey quickly increases in size, while gonadal development is inhibited for another 18 months to two years.

CONCLUSIONS:

(1) Ammocoetes of British lampreys can be selectively identified on the basis of shape, pigmentation and myomere counts.

(2) British lampreys of all three species require a water temperature of between 11 and 13°C for success-:ful spawning.

(3) *L. planeri* has a larval life of about five years.

(4) *L. fluviatilis* has a larval life of approximately 6 years.

(5) *P. marinus* has a larval life of about 5 years and 8 months.

(6) All three species begin metamorphosis in August or September.

(7) *P. marinus* larvae complete metamorphosis in about four months.

(8) *L. planeri* larvae and *L. fluviatilis* larvae require seven months to complete metamorphosis.

(9) Rate of feeding, hence of growth, in all three species studied is dependent, at least mainly, on the water temperature.

(10) Lamprey larvae do not feed at water temperatures below 11°C.

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A key to ammocoetes of British lampreys

A1 - A maximum of 58 trunk myomeres - L. fluviatilis.

$A_2$ - A minimum of 60 trunk myomeres.

$B_1$ - Area of caudal fin largely of a milky transparency, wi many small dark melanophores in a narrow margin outlining the posterior tip of the body - L. planeri.

$B_2$ - Area of caudal fin sprinkled widely with grey melanophores - P. marinus.

The specimens are to be placed in a narrow tes-tube and the myom counts made with a lens.

| Species | Range in number of myomeres | Average number of myomeres | Number of ammocoetes |
|---|---|---|---|
| L. planeri | 62-69 | 65.2 | 6,043 |
| L. fluviatilis | 51-58 | 54.4 | 4,102 |
| P. marinus | 61-69 | 67.2 | 1,184 |

Macdonald obtains pure samples of L. planeri from the Field Station Burn, river lamprey ammocoetes from the Fruin River, and marine lampreys from the Inler Burn, a tributary of the River Leven, Dumbartonshire, about 1 km. from Loch Lomond.

ABSTRACT OF THESIS: A STUDY OF CERTAIN ASPECTS OF AMMOCOETE BIOLOGY

By Theodore H. MacDonald

Three species of lamprey are found in Britain; the Sea Lamprey (*Petromyzon marinus*), the River Lamprey (*Lampetra fluviatilis*) and the Brook Lamprey (*Lampetra planeri*). Although the adult stages of these species can readily be distinguished because of their great differences in size, colour and dentition, the larvae, or ammocoetes, of all three species look superficially alike.

The first part of the thesis, therefore, concerns itself with the selective identification of the ammocoetes of the three species concerned. Close examination of more than 11,000 ammocoetes collected from streams in England, Scotland and Wales, indicated that the following are useful taxonomic characteristics:

(a) Pigmentation and shape of the precursor of the tongue.

(b) Pigmentation and shape of the caudal fin.

(c) Pigmentation of the branchial region.

(d) Number of trunk myomeres.

The pigmentation of ammocoetes is subject to some

variation in intensity within the same species. For instance, ammocoetes of L. planeri tend to be darker in the winter than they are in the summer within any given small area. Likewise, during the summer months, ammocoetes taken from streams in the north of Scotland are considerably darker than ammocoetes of the same species from streams in southern England.

As a suitable prelude to experimental and field studies made on the rates of growth in ammocoetes, the spawning requirements and embryological development of all three species is discussed.

This leads into, and forms a foundation for, the third part of the thesis, i.e. growth in ammocoetes. Samples of ammocoetes were taken from various streams, and the number of year classes making up the larval life of each species were estimated. Ten ammocoetes of each size-class of each species were then reared for one year in the laboratory in order to verify the field data. The conclusions arrived at in the field were thus upheld in the laboratory and were as follows:

(a) L. planeri has a larval life of about five years. Metamorphosis begins in August or September, and is completed in about seven months.

(b) L. fluviatilis has a larval life of approximately six years. Metamorphosis begins in August or September, and is completed in about seven months.

(c) P. marinus larvae complete metamorphosis in approximately five years and eight months. As in the other two species, metamorphosis commences in late summer but is completed in only four months or so.

A series of laboratory experiments in which ammocoetes were reared under artificially high and low temperatures, indicated that the rate of increase in weight and length, is closely correlated with the water temperature. The data make it apparent that ammocoetes do not feed during the winter months (the critical water temperature being about 11°C).

Year Class analysis of a sample of L. planeri ammocoetes taken in the Canadian arctic indicates that, when the winters are long and hence the feeding season reduced, a greater number of years of larval life are required before the ammocoetes undergo metamorphosis.